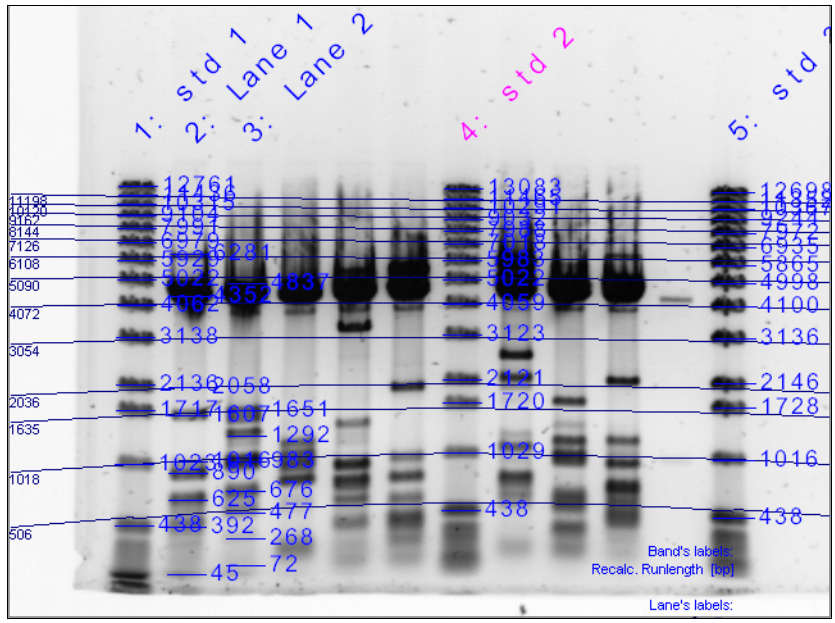
raytest

AIDA 1D Evaluation for Windows

User's Manual



Part Number 2003 01 1.1

© 2003 raytest Isotopenmeßgeräte GmbH, Benzstr. 4, D-75334 Straubenhardt, Germany.

All rights reserved. This product or document is protected by copyright and distributed under licenses restricting its use, copying, distribution and decompilation. No part of this product or document may be reproduced in any form by any means without prior written authorization of raytest and its licensors, if any.

RESTRICTED RIGHTS LEGEND: Use, duplication, or disclosure by the government is subject to restrictions as set forth in subparagraph (c)(1)(ii) of the Rights in Technical Data and Computer Software clause at DFARS 252.227-7013 and FAR 52.227-19.

The product described in this manual may be protected by one or more U.S. patents, foreign patents, or pending applications.

TRADEMARKS
AIDA, AIDA Image Analyzer,AIDA Annotation, AIDA AIDA Array Metrix, AIDA Array Compare, AIDA Multi Labeling, AIDA 1D Evaluation, AIDA 1D Densitometry, AIDA 2D Densitometry, AIDA Easy Array, AIDA Whole Body Autoradiography, AIDA 1D Thin Layer Chromatography and AIDA 2D Thin Layer Chromatography are trademarks or registered trademarks of raytest in the United States and may be protected as trademark in other countries.

All other product, service, or company names mentioned herein are claimed as trademarks and trade names by their respective companies.

THIS PUBLICATION IS PROVIDED "AS IS" WITHOUT WARRANTY OF ANY KIND, EITHER EXPRESS OR IMPLIED, INCLUDING, BUT NOT LIMITED TO, THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT.

THIS PUBLICATION COULD INCLUDE TECHNICAL INACCURACIES OR TYPOGRAPHICAL ERRORS. CHANGES ARE PERIODICALLY ADDED TO THE INFORMATION HEREIN, THESE CHANGES WILL BE INCORPORATED IN NEW EDITIONS OF THE PUBLICATION. raytest MAY MAKE IMPROVEMENTS AND/OR CHANGES IN THE PRODUCT(S) AND/OR THE PROGRAMS(S) DESCRIBED IN THIS PUBLICATION AT ANY TIME.

Contents

1 Introduction 5

Overview 5

Starting 1D Evaluation 6

2 Using 1D Evaluation 9

Data Windows in 1D Evaluation 9

Image Window in 1D Evaluation 10

Contextual Menu of the Image Window 11

Image Overlays 12

Profiles Window 16

Toolbar of the Profiles Window 25

Data Description 27

Contextual Menu of the Profiles Window (Graphics Pane) 30

Contextual Menu of the Profiles Window (Result Table) 34

Quantity Calibration Window 38

Contextual Menu of the Quantity Calibration Window 39

Runlength Calibration Window 42

Contextual Menu of the Runlength Window 43

Additional Toolboxes in 1D Evaluation 46

Lane & Peak Determination Toolbox 46

Lane Determination Pane 48

Baseline Determination Pane 50

Peak Determination Pane 51

Quantity Calibration Toolbox 52

Standard Pane 53

Calibration Curve Pane 55

Migration Distance Toolbox 56

Publishing Layout Toolbox in 1D Evaluation 58

Print Options for Profiles 59

Print Options for Quantity Calibration 60

Print Options for Runlength Calibration 61

Print 62

Preview 63
Export and Import 66
Exporting Templates 66
Exporting Profiles 67
Exporting 1D Report Table 68
Exporting Quantity Calibration 69
Exporting Runlength Calibration 70
Importing Profile Templates 72
Importing Quantity Calibration 73

3 Performing 1D Evaluations 75

Assigning Lanes and Bands 75
Assigning Migration Distance Standards 84
Assigning Quantitative Calibration Standards 87
Configuring the Evaluation Output 90

Introduction 1

Overview

Most biochemical applications utilize only one dimension as for example SDS protein gels, DNA agarose gels, thin layer chromatography and isoelectric focusing gels. These applications result in lanes originating from different samples that can be evaluated on the basis of their migration distance and signal intensity.

The AIDA 1D Evaluation module facilitates the analysis of 1D applications using quantification and migrations distance evaluation. For the lanes of a 1D application (e.g. an agarose gel of ethidium bromide stained DNA), rectangular densitometer windows can be defined to create a profile. The profile is the signal intensity at each point plotted against the length of the densitometer. The signal intensity can be integrated or averaged over the entire width of the densitometer. It is important to enclose the lane (each band) entirely with the densitometer because the bands in many applications vary in their signal intensity over their entire width. Encircling the entire band is especially important when using the integration option. The integrated signal intensity can be compared between lanes (relative comparison, e.g. of expression rates) or normalized by amount standards (e.g. 10, 20, 50, 100 ng of DNA) that are also present in standard lanes in the same image, or even in other images of the same series. The profiles can also be used to evaluate the molecular weight, fragment size, pI or RF values of the samples, depending on the employed separation method and sample. To that end the position of the bands are detected and the migration distance of the samples is compared to that of standards on the same image.

It is very important to understand and acknowledge the function of these evaluations to prevent pitfalls that will lead to results probably not reflecting the actual situation. Thus some suggestions for practical considerations for the sample preparation and image capturing will be given at the various evaluation section.

In general it should be noted that all comparisons of samples to standards should be validated. Thus it is for example not a good idea to evaluate the amount of protein A by using a standard of protein B, because in general various proteins stain different in coomassie and silver stains. This is also true for western blots of different proteins. However, the amount of the same protein in different lanes can be evaluated. On the other hand the evaluation of the fluorescence signal of ethidium bromide stained DNA samples

can be compared between any sample with acceptable variations (around 5 %). Also the evaluation of radioactive binding (e.g. in southern, northern or dot blots) yield accurate results.

It should also be kept in mind that the evaluations get more accurate with more standard values. Standards should be well scattered around the intensities or migration positions that are going to be examined. When in doubt it might be a good idea to check the reproducibility of 'known' results. Including and evaluating two fold dilutions of samples will also make quantitative estimations safer.

Starting 1D Evaluation

To select the 1D Evaluation mode:

1 Click the **Evaluation** button in the toolbar.

A dialog box appears offering you a list of available evaluation modes.

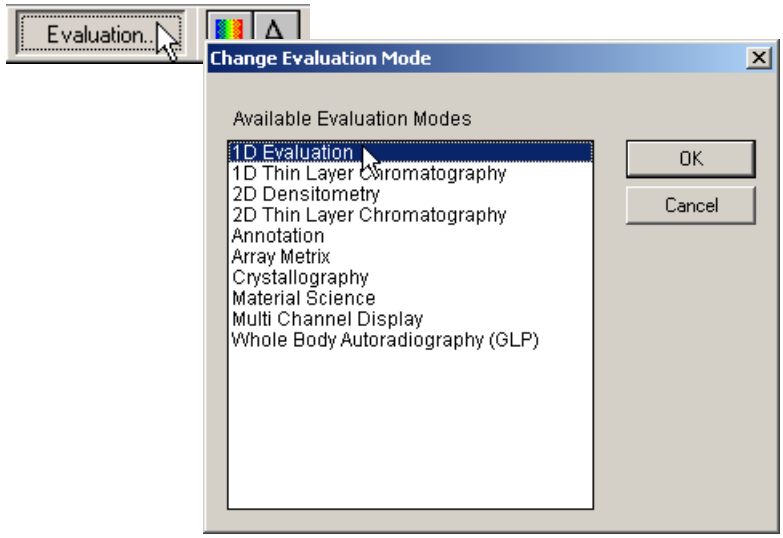


Figure 1-1 Changing Evaluation Mode

The list entries reflect the installed AIDA application modules.

2 Select the **1D Evaluation** list item und click **OK**.

Note – This evaluation mode is only available if you purchased the AIDA 1D Evaluation module.

Using 1D Evaluation 2

Data Windows in 1D Evaluation

In addition to the AIDA standard data windows **Image** and **Histogram** the following data windows are available in the **1D Evaluation** mode:

- Profiles
- Quantity Calibration
- Runlenght Calibration

You can access these data windows by choosing the appropriate entry (**Profiles**, **Quantity Calibration** and **Runlenght Calibration** respectively) from the **View** menu.

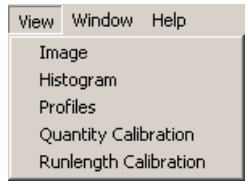


Figure 2-1 View Menu

Image Window in 1D Evaluation

All data are evaluated from the **Image** window. Although it might not always be on top, it is always open. The title bar of the **Image** window shows the full file path of the image.

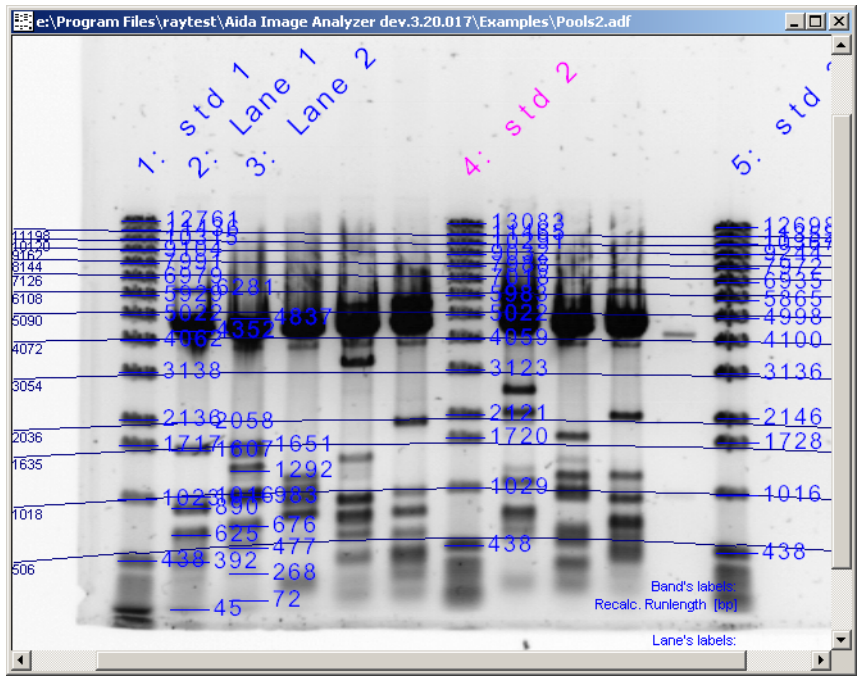


Figure 2-2 Image Window Example

You can copy the content of the **Image** window to the clipboard using the **Copy Content** command of the **Edit** menu. See the Export Image section in the AIDA Image Analyzer Basic Concepts and Features User's Manual for further explanation.

Contextual Menu of the Image Window

In 1D Evaluation, the contextual menu of the **Image** window looks slightly different depending on the type of overlay, you have chosen:

Lane Selection:
If you have selected one or more lanes, the contextual menu, that opens after right-clicking the respective object, provides you with the possible commands for this specific selection. These include commands for editing and labeling profiles (lanes).

Band Selection:
If you have selected a single band, the contextual menu, that opens after right-clicking the respective object, provides you with the possible commands for this type of selection. These include commands for editing and labeling profiles (lanes) as well as commands for editing peaks (bands). Note that if you select multiple bands, the peak editing command of the contextual menu is turned off.

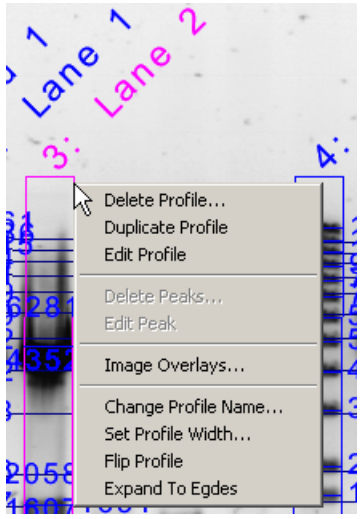


Figure 2-3 Contextual Menu (Lane Selected)

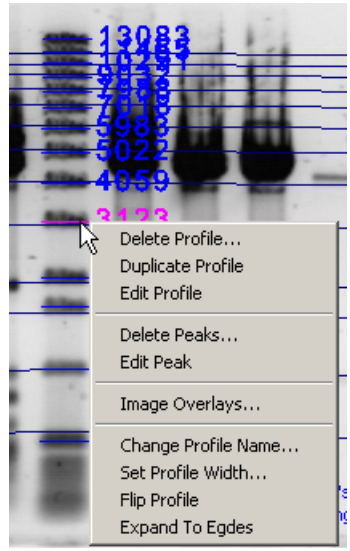


Figure 2-4 Contextual Menu (Band Selected)

The **Delete Profiles** and **Delete Peaks** commands are only executed for the selected lane(s) and selected peaks respectively.

With the **Duplicate Profile** commands you can insert another profile, identical to the currently selected one.

Edit Profile allows you to reshape the currently selected profile.

The currently selected peak area (band) can be re-defined with the **Edit Peak** command.

The **Image Overlays** command opens the **Image Overlays** dialog box described below.

Change Profile Name is used to change the name of the currently selected lane.

Set Profile Width is used to adapt the width of the densitometer windows drawn around the lanes.

Flip Profiles changes the densitometer orientation upside down and **Expand To Edges** expands the densitometer window beyond its narrow sides over the entire length or width of the image.

If no overlay is selected, only the basic editing functions of the standard contextual menu described in the AIDA Image Analyzer Basic Concepts and Features User's Manual will be accessible.

Image Overlays

You can specify the label type and font you want to apply to the overlays (lanes and bands) using the **Image Overlays** command of the **Options** menu.

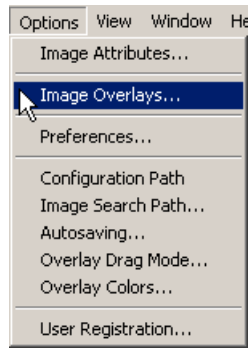


Figure 2-5 Options Menu: Opening the Image Overlays Dialog Box

After choosing **Image Overlays** from the **Options** menu, the **Image Overlays** dialog box opens allowing you to specify the labeling and marking of lanes and bands separately, using the **Lanes** and **Bands** tab respectively. In addition, you can specify marking and labeling for **Runlength Calibration**.

For **Lanes**, depending on the already performed evaluations, various labels can be selected by clicking the **Configure** button in the **Label** pane of the **Lanes** tab.

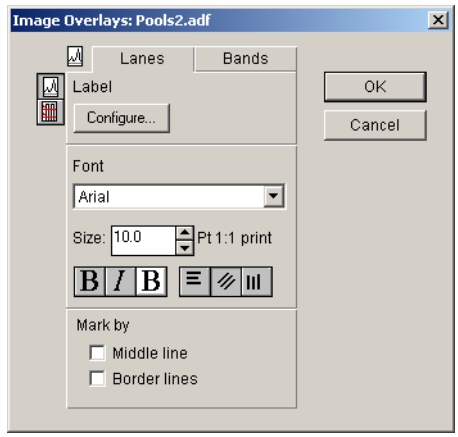


Figure 2-6 Image Overlays Dialog Box: Lanes Tab

This opens the **Columns Selection** dialog box, which provides you with a list of possible labels (e. g. lanes can be labeled by **Number**, **Profile** name or not at all.

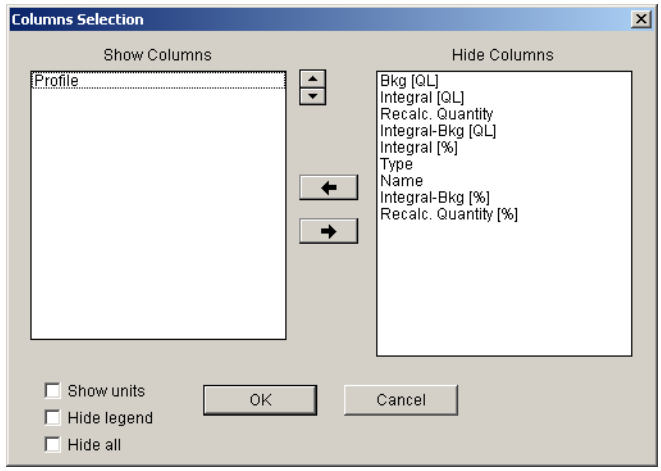


Figure 2-7 Columns Selection Dialog Box

After you have determined the type of labeling, you can set the font, font size, and font style (bold, italic, roman typeface on white background) as well as the rotation (0°, 45°, 90°) for the labels. The detected lanes can be surrounded by a frame (**Border Lines**) and tracked by a **Middle Line**.

Similar to the lanes, assigned peaks can be labeled using the **Configure** button of the **Label** pane on the **Bands** tab.

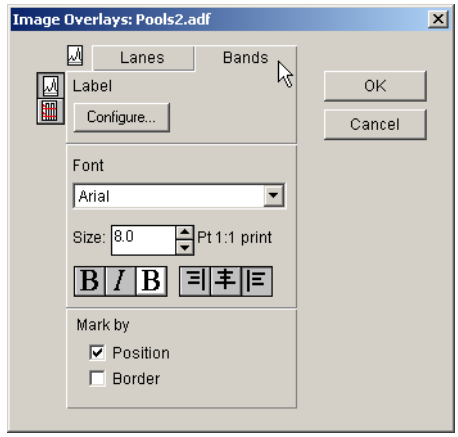


Figure 2-8 Image Overlays Dialog Box: Bands Tab

As for the lanes, use the **Font** pane of the dialog box to set the font options (font, font size, font style). Specify the position of label relative to the band marks (left, center, right) using the controls next to the font style selection buttons.

To insert marks for the peaks on the image, select the appropriate checkboxes in the **Mark By** part of the **Image Overlays** dialog box (**Position** and/or **Border**)

Besides the labeling for lanes and bands, the **Image Overlays** dialog box allows you to configure the display of runlength calibration marks and labels:

Just click the second icon on the toolbar to switch to **Runlength Calibration** pane.

Figure 2-9 Image Overlays Dialog Box: Toolbar

To turn on the drawing of standard lines on the image, click to select the **Draw Standard Lines** option.You can change the font size for the labels using the **Label Size** combo box.

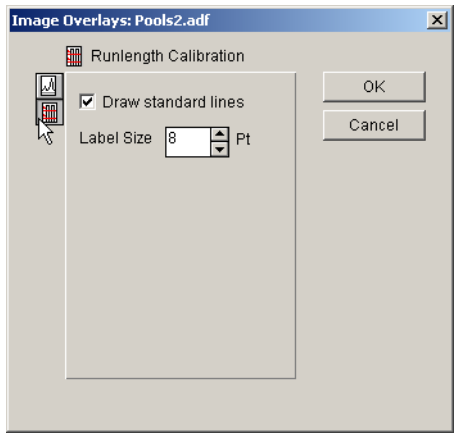
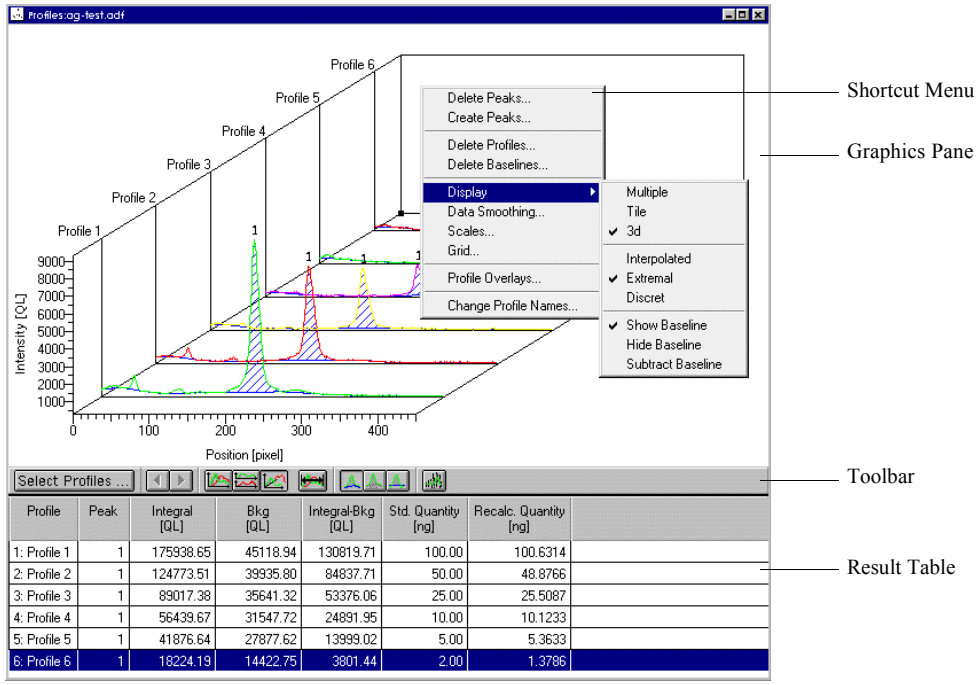


Figure 2-10 Image Overlays Dialog Box: Runlength Calibration Tab

Profiles Window

The **Profiles** window is bipartite, the upper pane displays the change of intensity over the length of the densitometer window, integrated or averaged (as selected) over the width of the densitometer window, the lower one contains a table, listing the standards, their given and recalculated values etc.



Profile	Peak	Integral [QL]	Bkg [QL]	Integral-Bkg [QL]	Std. Quantity [ng]	Recalc. Quantity [ng]
1: Profile 1	1	175938.65	45118.94	130819.71	100.00	100.6314
2: Profile 2	1	124773.51	39935.80	84837.71	50.00	48.8766
3: Profile 3	1	89017.38	35641.32	53376.06	25.00	25.5087
4: Profile 4	1	56439.67	31547.72	24891.95	10.00	10.1233
5: Profile 5	1	41876.64	27877.62	13999.02	5.00	5.3633
6: Profile 6	1	18224.19	14422.75	3801.44	2.00	1.3786

Figure 2-11 Profiles Window Example

In the graphics pane of the **Profiles** window, the displayed range of both axis can be zoomed:

To select a display range on an axis:

1 Choose the **Select** tool from the toolbar.

Figure 2-12 Select Tool on the Main Toolbar

2 Click at the smallest value of interest on the x-axis and drag to the biggest value of interest on the same axis with the left mouse button pressed.

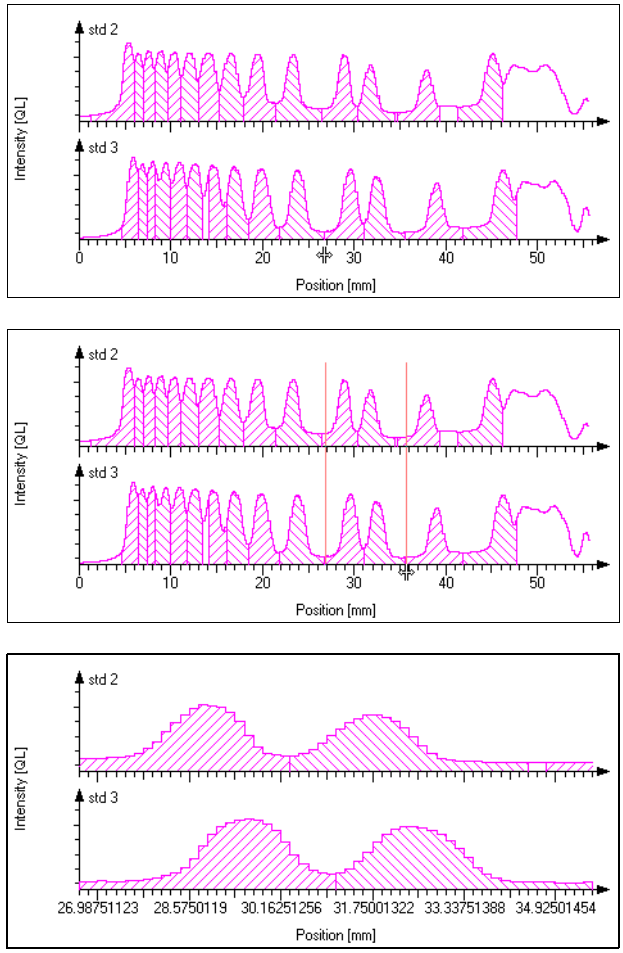


Figure 2-13 Zooming X-Axis

3 Release the mouse button.

The scaling will change accordingly.

This procedure is also applicable in 3D display mode:

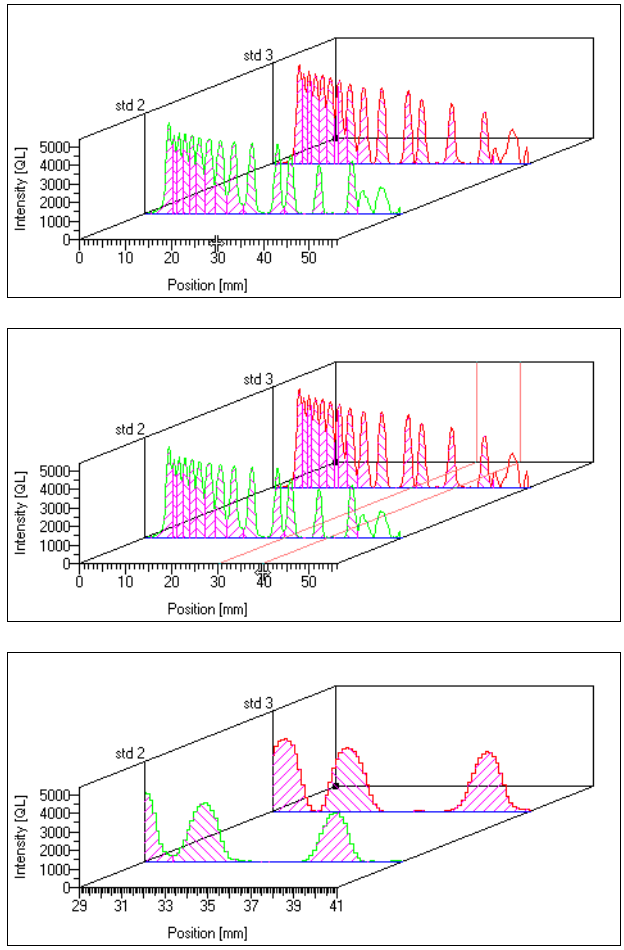


Figure 2-14 Zooming X-Axis in 3D Display Mode

To zoom out again, click the right mouse button when the ⇹ symbol is displayed. The maximal zoom-out factor is defined using the **Data Scaling** command (see below).

The same procedure can be applied to the y-axis.

To zoom into the Profiles window using the Zoom tool:

1 Click the Zoom tool on the toolbar.

Figure 2-15 Selecting Zoom Tool

2 Draw a rectangle around the area of the plot you want to see in more detail.

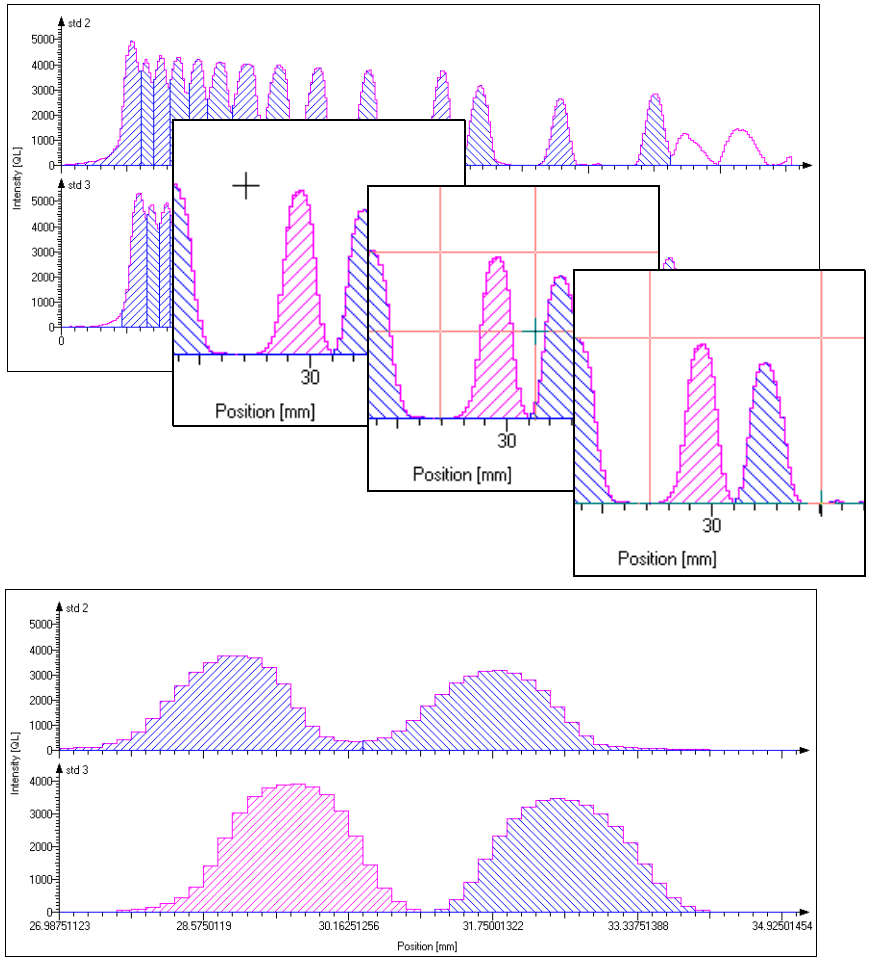


Figure 2-16 Zooming into Profiles Window

3 Release the mouse button.

4 Right-click to zoom out.

This procedure is also applicable in the 3D display mode, with the difference that you have to draw the rectangle on the back plane of the cuboid:

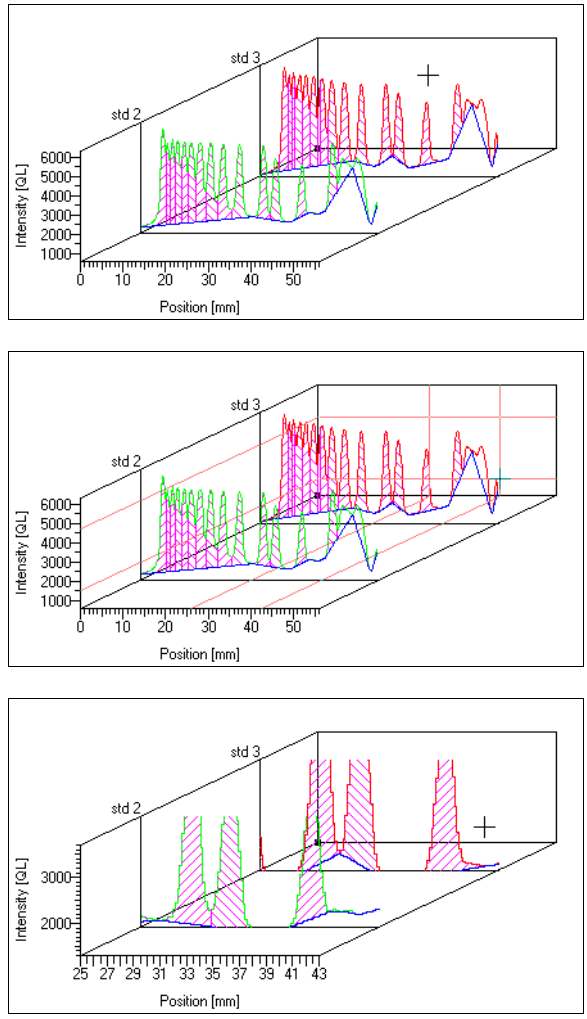


Figure 2-17 Zooming into Profiles Window in 3D Display Mode

To change your viewpoint in 3D display mode:

- Drag the axis origin with the mouse.

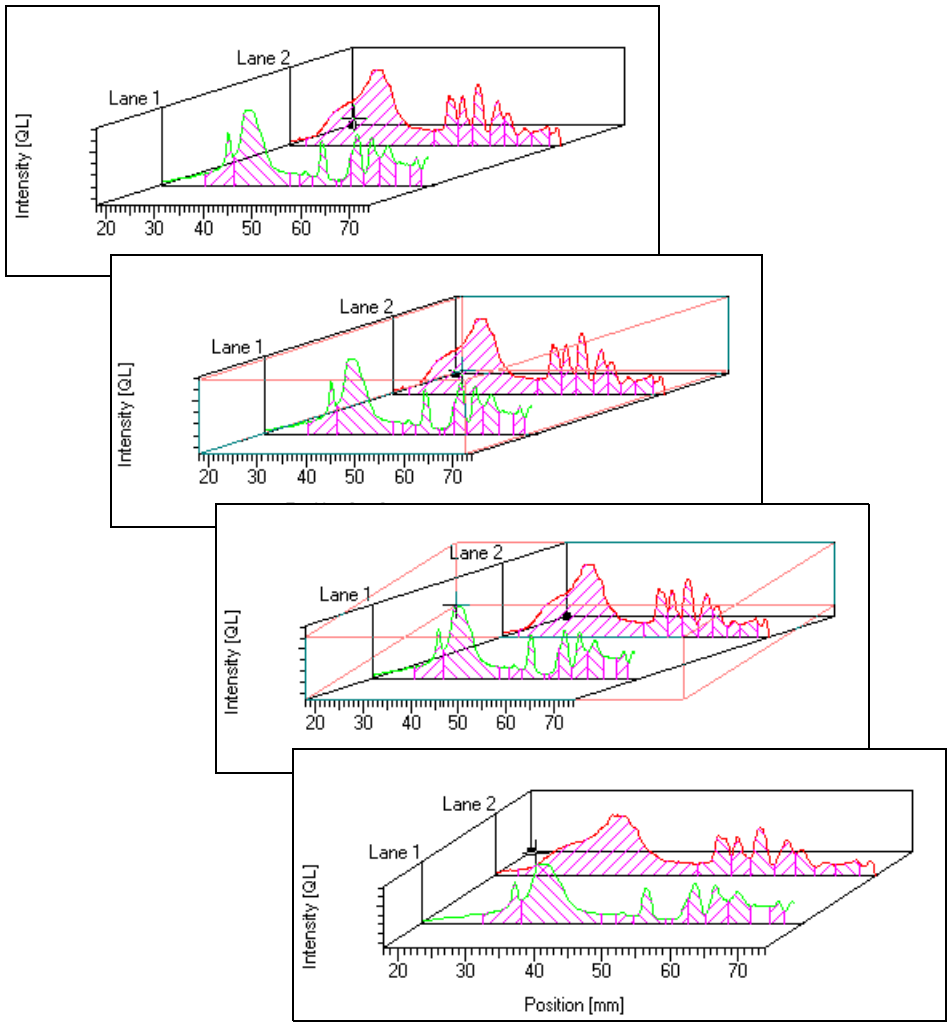


Figure 2-18 Changing 3D Viewpoint

To edit peaks:

1 Double-click the peak, whose borders or position you want to edit.
Vertical delimiter bars appear.

2 Drag the vertical delimiter bar.

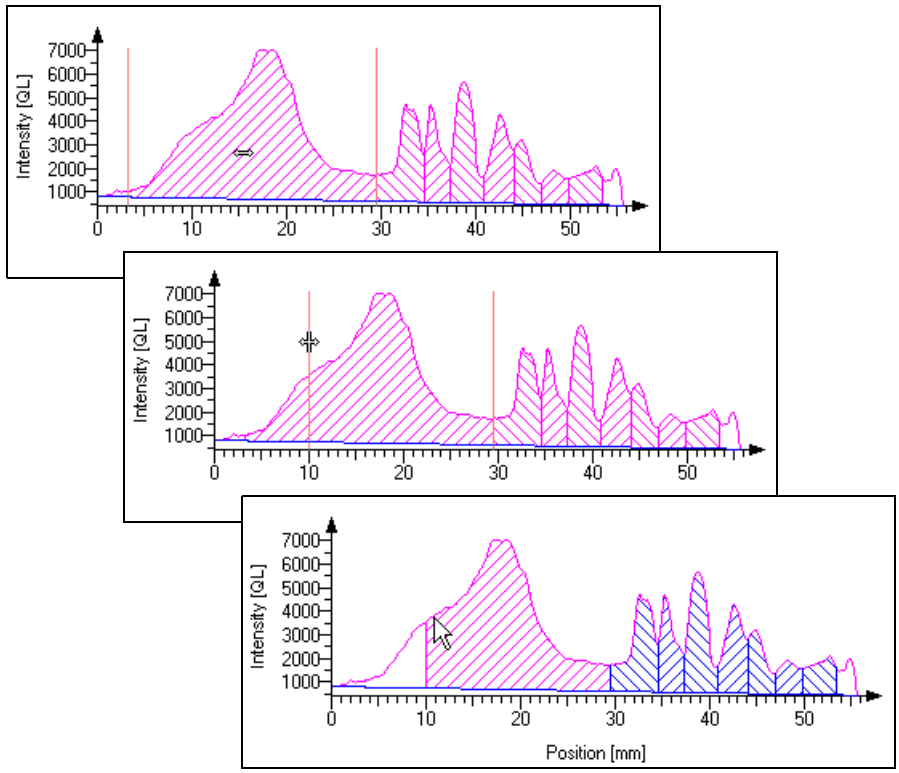


Figure 2-19 Editing Peaks

3 Right-click to confirm the changes.

To delete a peak:

1 Right-click on the hatched area indicating the peak's domain.

2 Choose **Delete Peaks** from the contextual menu.

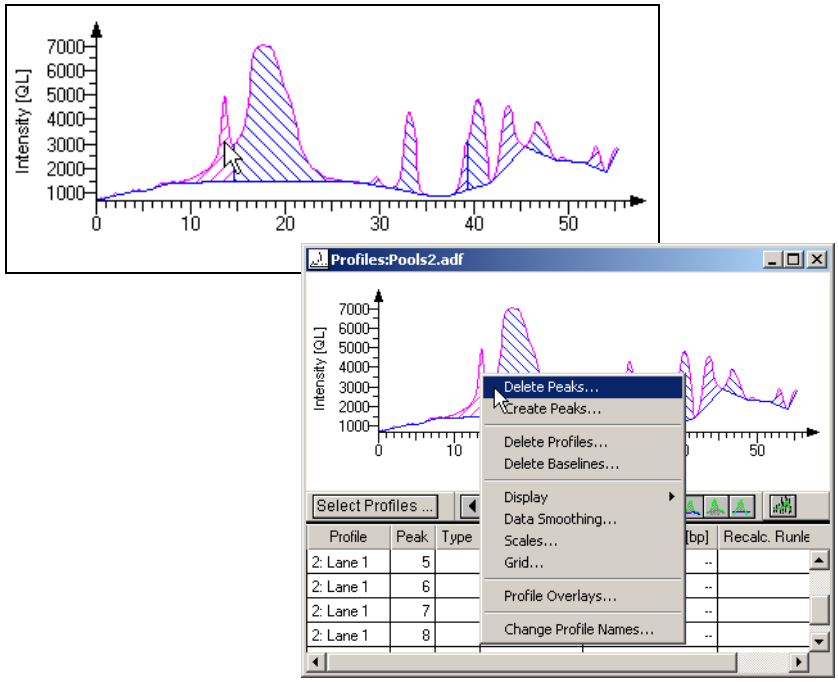


Figure 2-20 Selecting peak and Opening Contextual Menu

A dialog box opens, prompting you to confirm the command.

3 Click **OK** to delete the selected peak.

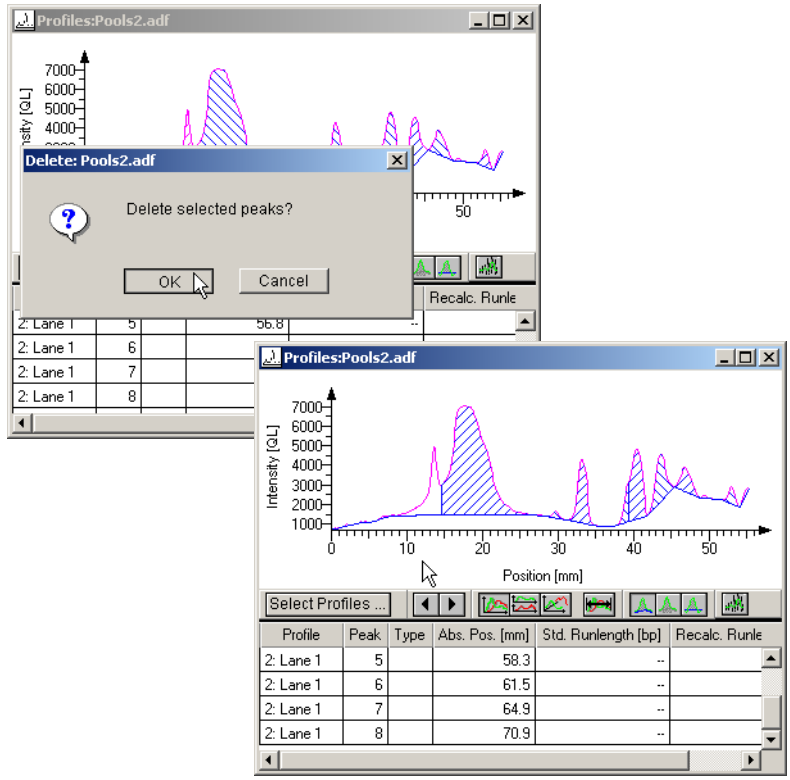


Figure 2-21 Confirming Deletion of Peak

Alternatively, you can delete peaks by first selecting the peaks in the **Image** window and pressing the DEL key on your keyboard.

Toolbar of the Profiles Window

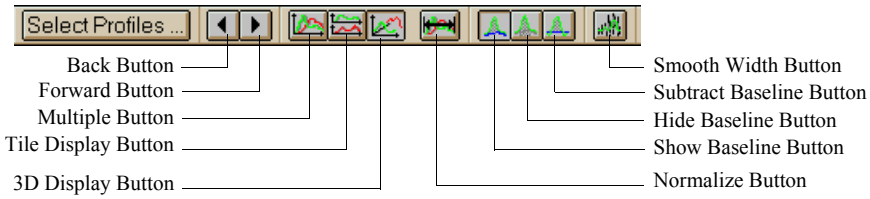


Figure 2-22 Profiles Window Toolbar

Using the **Select Profiles** button you can select any profile by its name. With the arrow buttons next to the **Select Profiles** button you can browse forward or backwards through the available profiles. The three buttons in the middle provides you with different display possibilities for the graphical output:

Multiple displays multiple curves for selected items in a single two-dimensional co-ordinate system, using different colors for each.

3D shows all curves for selected items in a single three-dimensional co-ordinate system.

Tile uses a separate 2D co-ordinate system for each curve of a selected item.

The **Normalize** button is used to normalize all displayed profiles according to the determined migration distance.

The **Show Baseline**, **Hide Baseline**, **Subtract Baseline** buttons are used to display the determined baseline, hide the baseline and subtract everything below the baseline, respectively. This function is only available if a baseline has been set in the **Band & Peak Determination** toolbox.

The **Smooth Width** button opens a dialog box to enter the smooth width for the curve display. A higher number will result in averaging over the given range of pixel, mm, inch, where the unit is set using the **Image Attributes** commands of the **Options** menu.

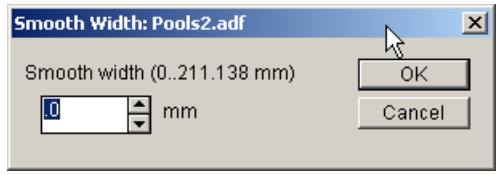


Figure 2-23 Smooth Width Dialog Box

The lower part of the **Profiles** window contains a table (henceforth *Result Table*), listing the standards, their given and recalculated values etc.

You can change the display ratio between the graphics and the table pane by dragging the delimiter line located on the bottom of the toolbar:

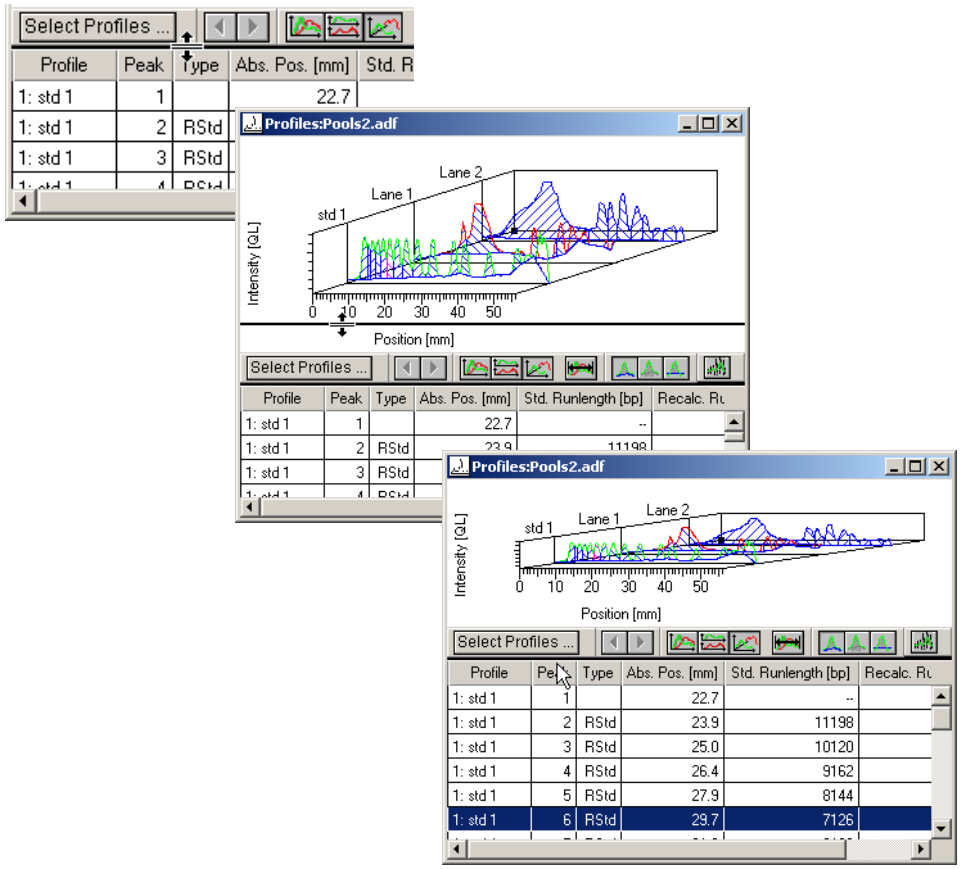


Figure 2-24 Changing the Display Ratio Between Graphics Pane And Result Table

Data Description

Available **Columns** in the **Result Table**:

Table 2-1 Columns Available in the Result Table of the Profiles Window

Column	Short Description
Profile	The index and name of the profile.
Peak	The number of the peak.
Type	The type of the peaks, e.g. QStd for Quantity Standards, RStd for Runlength Standard.
Name	The name of the peak as given by the user.
Rel. Pos. [*length unit*]	The position of the peak in the profile.
Abs. Pos. [*length unit*]	The position of the band in the image measured from the edges of the image.
Start Pos. [*length unit*]	The start position of the peak in the profile.
End Pos. [*length unit*]	The end position of the peak in the profile.
Width [*length unit*]	The width of the peak.
Height [*signal unit*]	The maximum height of the peak.
Integral [*signal unit*]	The integrated peak intensity.
Bkg [*signal unit*]	The integrated background intensity.
Bkg [%]	The percentage of the peak background in comparison to the **Sum** of all peak backgrounds, or to the value in the row which is 'Set to 100 %', if defined.
Integral-Bkg [*signal unit*]	The background-corrected integral of the peak.
Integral [%]	The percentage of the peak integral in comparison to the **Sum**., or to the value in the row which is 'Set to 100 %', if defined.
Integral-Bkg [%]	The percentage of the peak integral reduced by the background intensity in comparison to the **Sum**, or to the value in the row which is 'Set to 100 %', if defined.

signal unit and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

quantity unit and *runlength unit* = unit of measurement, specified by the user through quantity and runlength calibration respectively

Table 2-1 Columns Available in the Result Table of the Profiles Window

Column	Short Description
Std. Quantity [*quantity unit*]	The quantity of the peak, as entered for the calculation of the standard curve.
Recalc. Quantity [*quantity unit*]	A calculation of the peak quantity, based on the current calibration curve.
Recalc. Quantity [%]	The percentage of the peak quantity in comparison to the **Sum** of all peak quantities, or to the value in the row which is 'Set to 100 %', if defined.
Std. Runlength [*runlength unit*]	The runlength standard value of the peak, as entered. Depending on the method for the runlength measurement, this is the standard for the RF value, Fragment Size, Molecular Weight or pI Value.
Recalc. Runlength [*runlength unit*]	A calculation of the peak runlength based on the current runlength curve. Depending on the method for the runlength measurement, this is the standard for the RF value, Fragment Size, Molecular Weight or pI Value.

signal unit and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

quantity unit and *runlength unit* = unit of measurement, specified by the user through quantity and runlength calibration respectively

The following additional rows can be found or created exclusively in the **Region Report**:

Table 2-2 Additional Columns in the Region Report

Sum	Displays the scientifically correct sum for the selected column.
Ttl (Total)	Sum over the entire profile.
Remainder	Ttl – Sum

Numbers in *italics* indicate that the value was interpolated over the end of the defined standard curve and is therefore only representing a tendency, not a result. When a field is not relevant for a profile a dash [-] is displayed. The numbers can be formatted as outlined below.

You can select the number and type of columns you want to display using the contextual menu of any column in the window (see below).

To change the column order:

1. Click the heading of the column to be moved.
2. Press the mouse button and drag to the desired position in the table.
3. Release the mouse button.

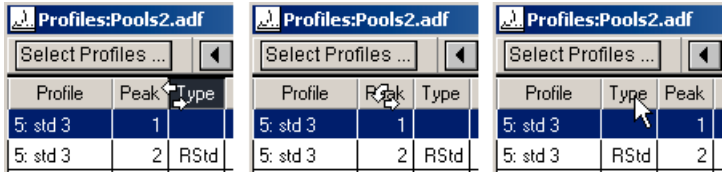


Figure 2-25 Changing Column Order

To change the column width:

1. Position the mouse pointer over the border of the column whose width you want to change.
2. Click and drag the border in the appropriate direction.
3. Release the mouse button:

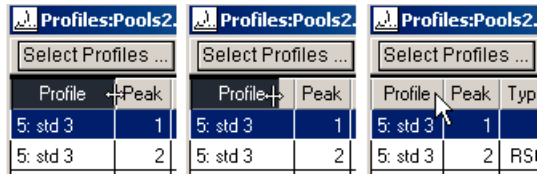


Figure 2-26 Changing Column Width

Contextual Menu of the Profiles Window (Graphics Pane)

Right-clicking on the **Profiles** window's graphics pane opens a contextual menu, providing you with commands for deleting/creating peaks, deleting profiles/baselines as well as several display settings (see below).

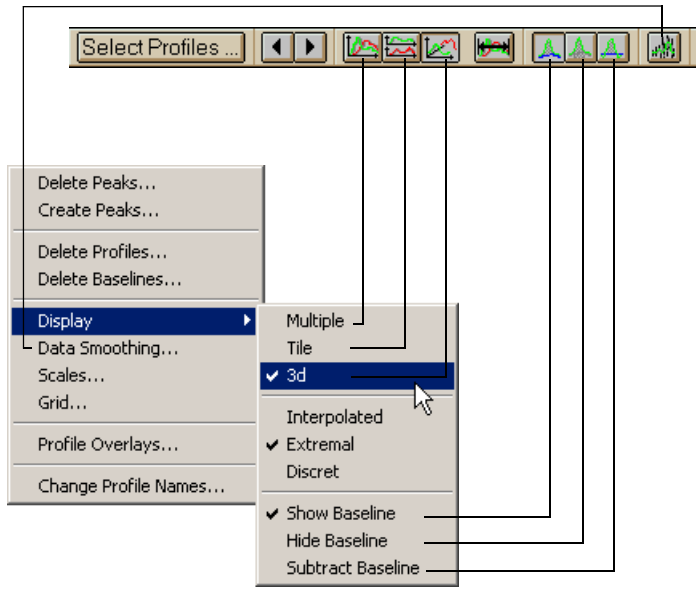


Figure 2-27 Contextual Menu of the Profiles Window's Graphics Pane

From the submenu of the **Display** command you can specify the appropriate display mode (**Multiple**, **Tile** and **3d** explained earlier) as well as how the data should be displayed: You can choose between the **Discrete** display of data points (pixels) in the profile, the display of **Extremal** data (representing the maximum value for all points between the determined data points), or the display of **Interpolated** curves between data points. Further, you can specify to show or hide the baseline using the **Show Baseline** or **Hide Baseline** command respectively. In addition you can choose to subtract the baseline from the data by selecting **Subtract Baseline**.

With the **Data Scaling** command you can configure the graphical output. The **Minimum** and **Maximum** value of the x- (**Position**) and y-axis (**Intensity**) can be selected together with a logarithmic representation for y-axis. Both axis can be set to **Automatic** scaling using the appropriate checkboxes. The **3D View Point** settings are the numerical representation of the change in 3D views as described above.

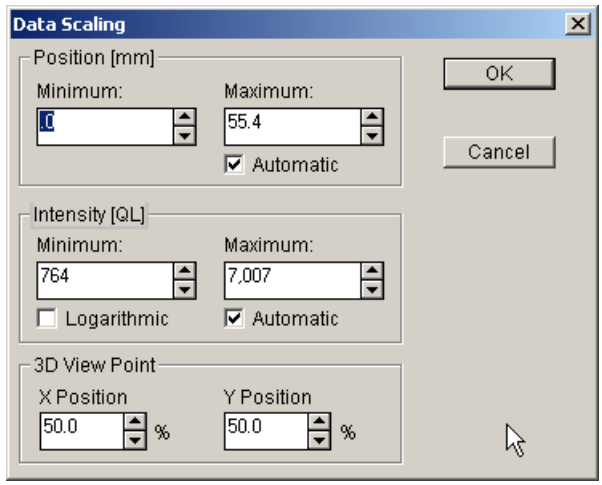


Figure 2-28 Data Scaling Dialog Box

The **Data Smoothing** function is used to smoothen the curve.

Figure 2-29 Smooth Width Dialog Box

The **Grid** command opens the **Grid** dialog box, which allows you to lay grids over both axis that are an extension of either the big or the small ticks on the axes.

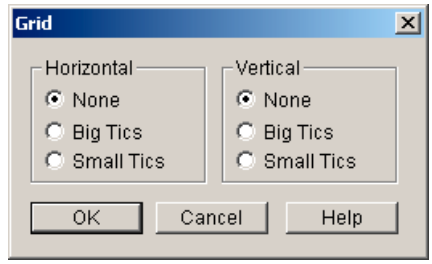


Figure 2-30 Grid Dialog Box

The **Profile Overlays** command opens a dialog box that allows you to select various labels for the peaks, together with their font, font size, font style and rotation angle. The display of peak **Area** and / or **Brackets** (indicating the bands' borders) can be selected. To turn off the marking of the peaks on the graphics display, click to deselect the **Draw Peaks** option.

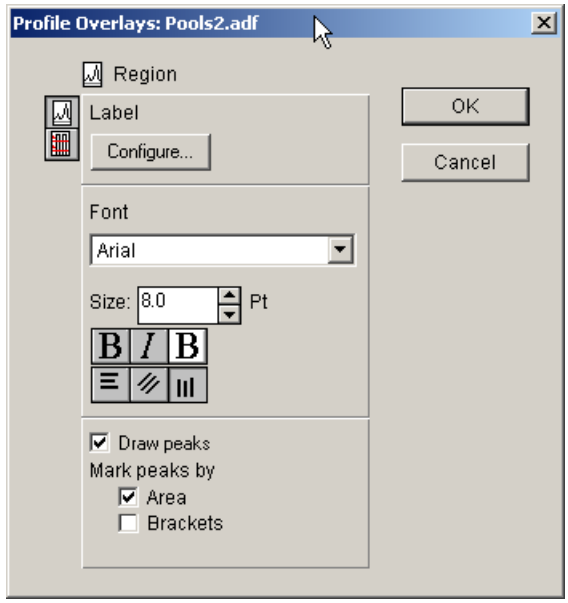


Figure 2-31 Profile Overlays Dialog Box (Label Pane)

The location of the migration distance standards can be drawn into the profile by clicking the **Draw Standard Lines** option on the **Runlength Calibration** pane. Further you can specify the font size of the labels by entering a value in the **Label Size** combo box.

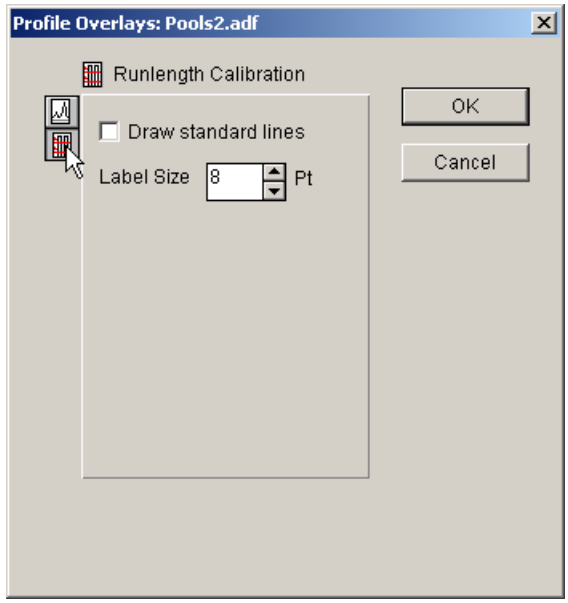


Figure 2-32 Profile Overlays Dialog Box (Runlength Calibration Pane)

With the **Change Profile Names** command you can re-name the densitometer windows and the resulting profiles according to your preferences. The names will appear in all result tables and image menus.

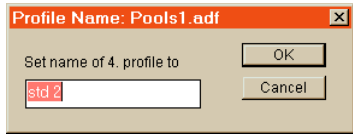


Figure 2-33 Profile Name Dialog Box

Contextual Menu of the Profiles Window (Result Table)

Each column in the **Result Table** has a contextual menu attached. To open the contextual menu, right-click on the column heading (gray area).

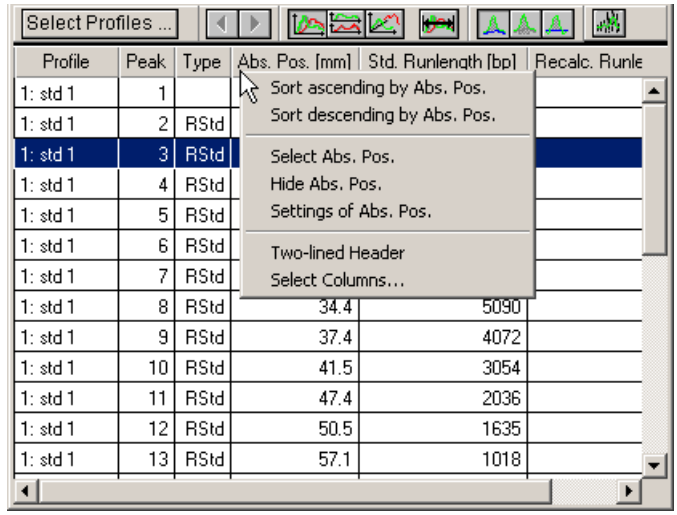

Select Profiles ...
Profile
Peak
Type
Abs. Pos. [mm]
Std. Runlength [bp]
Recalc. Runle
Sort ascending by Abs. Pos.
Sort descending by Abs. Pos.
Select Abs. Pos.
Hide Abs. Pos.
Settings of Abs. Pos.
Two-lined Header
Select Columns...
1: std 1 1
1: std 1 2 RStd
1: std 1 3 RStd
1: std 1 4 RStd
1: std 1 5 RStd
1: std 1 6 RStd
1: std 1 7 RStd
1: std 1 8 RStd 34.4 5090
1: std 1 9 RStd 37.4 4072
1: std 1 10 RStd 41.5 3054
1: std 1 11 RStd 47.4 2036
1: std 1 12 RStd 50.5 1635
1: std 1 13 RStd 57.1 1018

Figure 2-34 Contextual Menu of the Profiles Window's Result Table

With the first two commands of the contextual menu, you can sort the table of the **Result Table** by any value column in ascending order (A to Z or zero to 9) or descending order (Z to A or 9 to zero). Just choose the appropriate menu item (**Sort Ascending by**/**Sort Descending by**).

The **Select**/**Unselect** commands of the contextual menu are used to select/deselect a column to export. The column heading of selected columns are highlighted.

To hide a column, choose **Hide** command from its contextual menu. You can recover hidden columns using the **Select Columns** command (see below).

To specify the decimal digits for the value output in a column, choose **Settings of** from the contextual menu and enter the value for the decimal digits in the dialog box displayed.

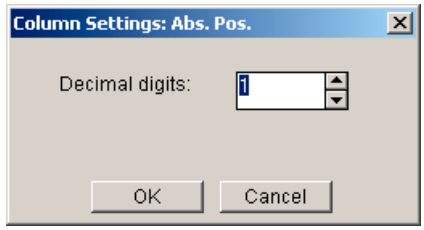


Figure 2-35 Setting Output Format for Columns

Use the **One-lined Header**/**Two-lined Header** command to reduce or expand the column heading by one line (the second line indicates the units of measurement).

To select/deselect the columns that should be displayed in the Result Table:

1. Choose **Select Columns**.

   A dialog box appears displaying two lists (**Show Columns**/**Hide Columns**).

2. Select the columns you want to show or hide and click the appropriate arrow symbol to transfer the items between the two lists.

3. To deselect a particular item, just click it again.

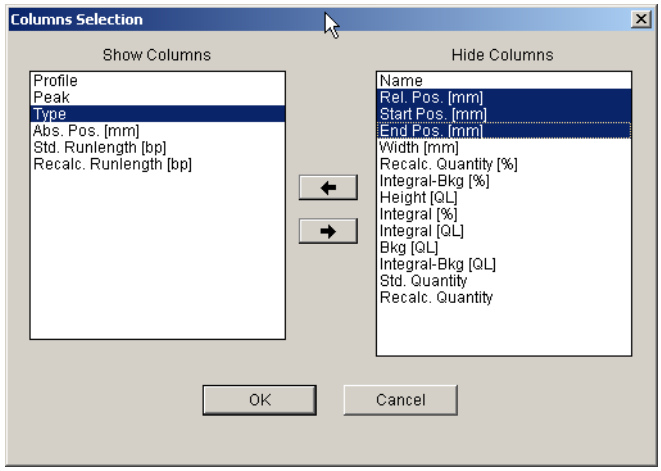


Figure 2-36 Columns Selection Dialog Box

In addition, each row of the table has a contextual menu attached, which includes commands for displaying special row types like **Sum**, **Total**, **Reminder** as well as for changing profile names.

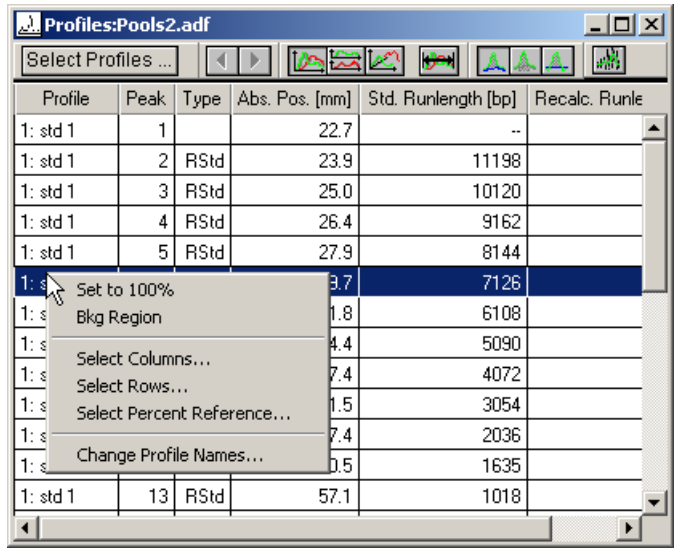


Figure 2-37 Contextual Menu of a Result Table Row

Set to 100 % will set the value in the active row to 100 % instead of using the **Sum** value as 100% and recalculate the other values accordingly.

To select the columns that should be displayed in the Result Table, choose the **Select Columns** command (see above).

Using the **Select Rows** command, you can choose four special row types to be displayed in the Result Table. In the dialog box that appears, click to select or deselect the appropriate options. Clicking **Total**, **Sum**, and/or **Remainder** inserts the respective row(s) into the table. **Separator** displays an extra separator row showing the name of the profile.

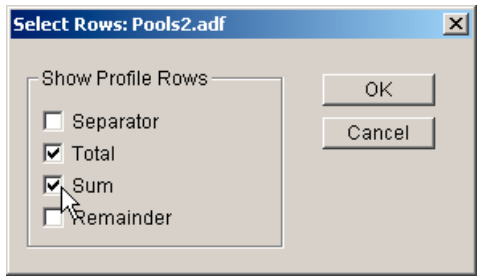


Figure 2-38 Select Rows Dialog Box

Select Percent Reference: This command allows you to set the reference value for the percentage calculation. This can be either a distinguished peak defined by the **Set to 100 %** command of the table contextual menu (see above), the value for the total profile, or the **Sum**. The later two are set via the following dialog box.

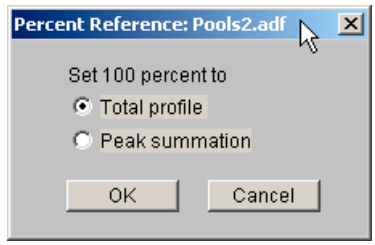


Figure 2-39 Percent Reference Dialog Box

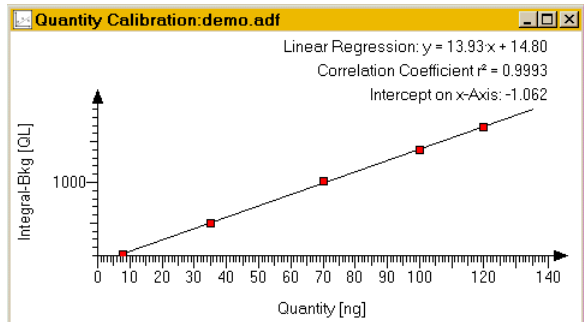
The **Change Profile Names** command allows to re-name the densitometer windows and resulting profiles according to your preferences. The names will appear in all result tables and image menus.

Figure 2-40 Profile Name Dialog Box

Quantity Calibration Window

To open the Quantity Calibration window:

- Choose **Quantity Calibration** from the **View** menu, *or*
- Double-click the miniaturized calibration curve display on the **Quantity Calibration** toolbox.


Quantity Calibration:demo.adf
Linear Regression: y = 13.93·x + 14.80
Correlation Coefficient r² = 0.9993
Intercept on x-Axis: -1.062
Integral-Bkg [QL]
1000
0 10 20 30 40 50 60 70 80 90 100 120 140
Quantity [ng]

Profile	Peak	Integral-Bkg [QL]	Quantity Std. [ng]	Quantity Recalc. [ng]
1: Standa	9	1675.8	120.0	119.25
1: Standa	11	1402.9	100.0	99.66
1: Standa	13	1019.2	70.0	72.11
11: Profile	1	494.6	35.0	34.44
11: Profile	2	119.9	8.0	7.54

Figure 2-41 Quantity Calibration Window Example

The **Quantity Calibration** window is bipartite, the upper pane displays the curve of the calibration function, the underlying function, the correlation coefficient and the x-axis intercept. The lower one contains a table, listing the standards, their given and recalculated standard values, so you can easily evaluate their differences. This can be useful to estimate the error for a certain amount calculated for an unknown band.

The graphical representation can be adapted using the contextual menu attached. For a more detailed view of the plot, you can activate the AIDA standard zoom functions described above.

The table can be exported as a text file or copied into the clipboard.

Additional columns can be displayed. Just right-click on the table heading and choose the **Select Columns** command from the contextual menu, that appears. The **Columns Selection** dialog box opens, allowing you to select the desired columns from the list.

Contextual Menu of the Quantity Calibration Window

The contextual menu of the upper pane of the **Quantity Calibration** window contains two commands: **Scales** and **Grid**.

With the **Scales** command you can configure the graphical output. After clicking the **Scales** command, the **Data Scaling** dialog box opens. Here the **Minimum** and **Maximum** value of the x- (Concentration) and y-axis (Intensity) can be selected. In addition, you can turn on logarithmic representation and automatic scaling of either axis separately by clicking the appropriate option (**Logarithmic** and **Automatic**) below the **Minimum** and **Maximum** spin boxes.

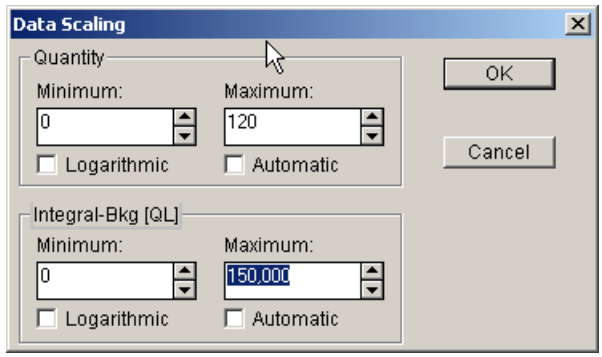


Figure 2-42 Data Scaling Dialog Box (Quantity Calibration)

The **Grid** command opens the **Grid** dialog box, which allows you to lay grids over both axis that are an extension of either the big or the small ticks on the axes.

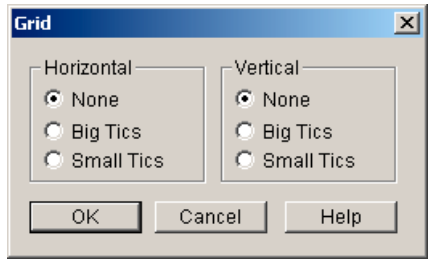


Figure 2-43 Grid Dialog Box

The table in the lower part of the **Quantity Calibration** window provides its own contextual menu:

The column headings are equipped with the standard contextual menus described in the “Profiles Window” section of this manual. If you right-click on any of the table rows, the row's contextual menu opens, allowing you to **Select Columns** for displaying or **Delete Standards** from the table.

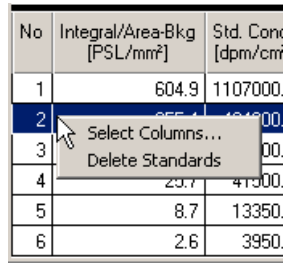


Figure 2-44 Quantity Calibration Window (Row Contextual Menu)

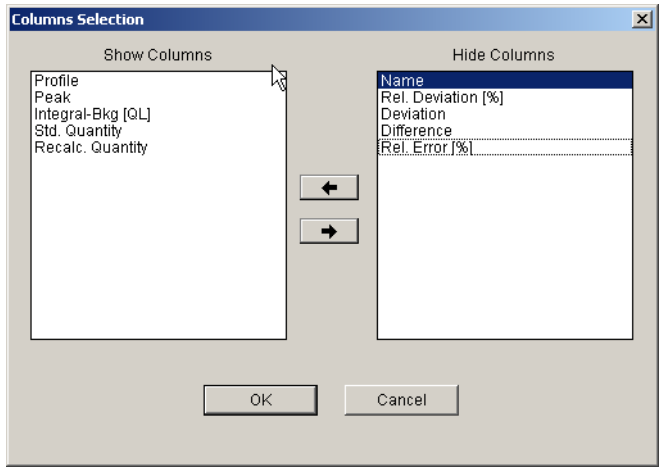


Figure 2-45 Selecting Columns to be Displayed

The following columns are available:

Table 2-3 Columns Available in the Quantity Calibration Window

Columns	Short Description
Profile	The index and name of the profile.
Peak	The number of the peak in the profile.
Name	The name for the peaks as given by the user.
Integral-Bkg [*signal unit*]	The background-corrected integral of the peak.
Std. Quantity [*quantity unit*]	The quantity of the peak, as entered for the calculation of the quantity standard curve.
Recalc. Quantity [*quantity unit*]	A calculation of the peak quantity, based on the current quantity calibration curve.
Difference [*unit*]	The difference without sign between the entered standard and the recalculated value.
Rel. Error [%]	The relative difference without sign of the entered standard and the recalculated value compared to the standard value (= Difference / Std. Quantity).
Deviation [*unit*]	The difference with sign between the entered standard and the recalculated value (Recalc. Quantity – Std. Quantity).
Rel. Deviation [%]	The relative difference with sign of the entered standard and the recalculated value compared to the standard value (= Deviation / Std. Quantity).

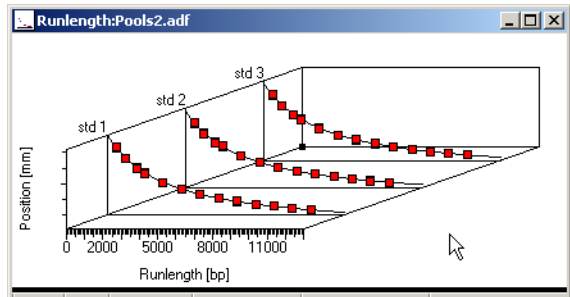
signal unit and *length unit* = units of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

quantity unit and *runlength unit* = unit of measurement, specified by the user through quantity and runlength calibration respectively

Runlength Calibration Window

To open the **Runlength** window, choose **Runlength Calibration** from the **View** menu.

Just as the **Quantity Calibration** window, the **Runlength** window is bipartite, the upper pane displays the curve of the calibration function for the different lanes. The graphical representation of the calibration function is shown for every lane. The lower part of the window contains the Result Table of the run length calibration function.



Profile	Peak	Position [mm]	Std. Runlength [bp]	Recalc. Runlength [bp]
1: std 1	2	23.8731	11198	11416
1: std 1	3	24.9944	10120	10284
1: std 1	4	26.3548	9162	9127
1: std 1	5	27.9265	8144	8017
1: std 1	6	29.7377	7126	6963
1: std 1	7	31.8423	6108	5964
1: std 1	8	34.3568	5090	5002
1: std 1	9	37.3976	4072	4081

Figure 2-46 Runlength Window Example

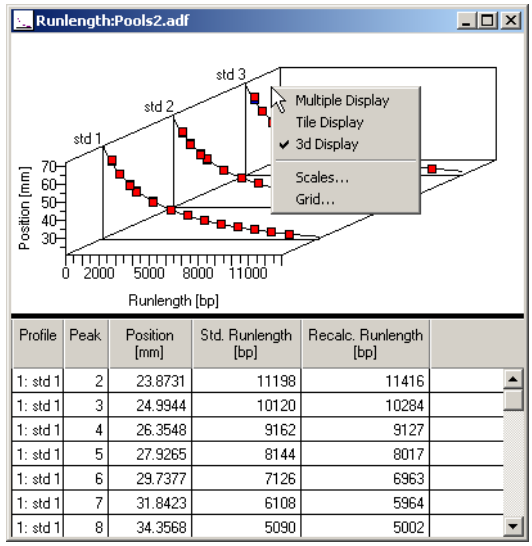
Again, the graph and adjacent table can be adapted by using the contextual menu. For a more detailed view of the plot, you can activate the AIDA standard zoom functions described above.

You can change the display ration between the graphics and the table pane by dragging the delimiter bar.

The standard curves of non-standard lanes are interpolated from the evaluated standard lanes. This requires multiple standard lanes on the gel that are correctly assigned.

Contextual Menu of the Runlength Window

Right-clicking on the **Runlength** window's graphics pane opens a contextual menu, providing you with commands for setting several display options (see below).



Profile	Peak	Position [mm]	Std. Runlength [bp]	Recalc. Runlength [bp]
1: std 1	2	23.8731	11198	11416
1: std 1	3	24.9944	10120	10284
1: std 1	4	26.3548	9162	9127
1: std 1	5	27.9265	8144	8017
1: std 1	6	29.7377	7126	6963
1: std 1	7	31.8423	6108	5964
1: std 1	8	34.3568	5090	5002

Figure 2-47 Runlength Window (With Contextual Menu)

Set the desired display mode using **Multiple Display**, **Tile Display** and **3d Display** (explained in the “Profiles Window” section of this manual)

The **Scales** command allows you to configure the graphical output. The **Minimum** and **Maximum** value of the x- (**Runlength**) and y-axis (**Position**) can be selected together with a logarithmic representation for both axis. In addition, both axis can be set to **Automatic** scaling using the appropriate checkboxes. The **3D View Point** settings are the numerical representation of the change in 3D views as described above.

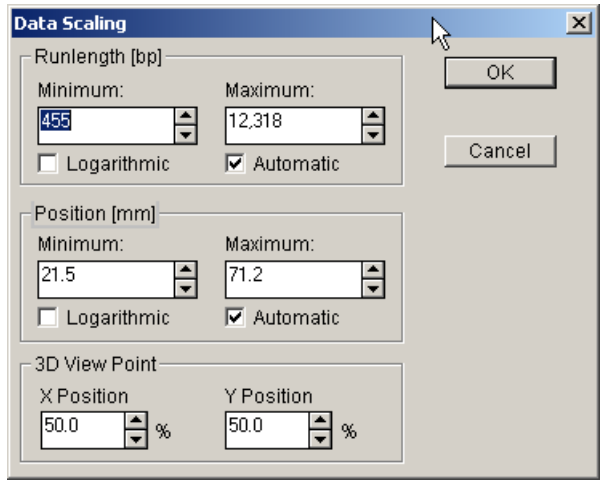


Figure 2-48 Data Scaling Dialog Box (Runlength Window)

The **Grid** command opens the **Grid** dialog box, which allows you to lay grids over both axis that are an extension of either the big or the small ticks on the axes.

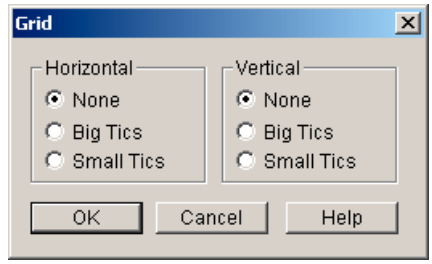


Figure 2-49 Grid Dialog Box

The table in the lower part of the **Runlength** window provides its own contextual menu:

The column headings are equipped with the standard contextual menus described in the “Profiles Window” section of this manual.

If you right-click on any of the rows in the table, the row's contextual menu opens, allowing you to **Select Columns** for displaying or **Change Profile Names**.

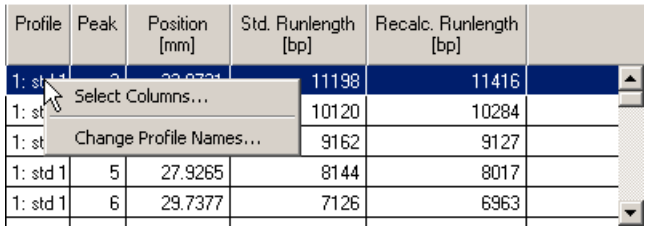


Figure 2-50 Table Row Contextual Menu

The following columns are available:

Table 2-4 Columns Available in the Runlength Window

Columns	Short Description
Profile	The index and name of the profile.
Peak	The number of the peak in the profile.
Name	The name for the peaks as given by the user.
Position [*length unit*]	The position of the peak in the profile.
Std. Runlength [*runlength unit*]	The runlength standard value of the peak, as entered. Depending on the method for the runlength measurement, this is the standard for the RF value, Fragment Size, Molecular Weight or pI Value.
Recalc. Runlength [*runlength unit*]	A calculation of the peak runlength based on the current runlength curve. Depending on the method for the runlength measurement, this is the standard for the RF value, Fragment Size, Molecular Weight or pI Value.
Difference [*runlength unit*]	The difference without sign between the entered standard and the recalculated value.
Rel. Error [%]	The relative difference without sign of the entered standard and the recalculated value compared to the standard value (= Difference / Std. Runlength).

length unit = unit of measurement, depending on the type of the imaging device used to obtain the image and the options set in the **Image Attributes** dialog box.

runlength unit = unit of measurement, specified by the user through runlength calibration.

Additional Toolboxes in 1D Evaluation

In addition to the standard AIDA toolboxes **Display Control** and **Publishing Layout**, the following toolboxes become available after selecting the **1D Evaluation** mode.

- Lane & Peak Determination
- Quantity Calibration
- Migration Distance

You can access these additional toolboxes from the **Evaluation** menu or by clicking the appropriate button on the toolbar.

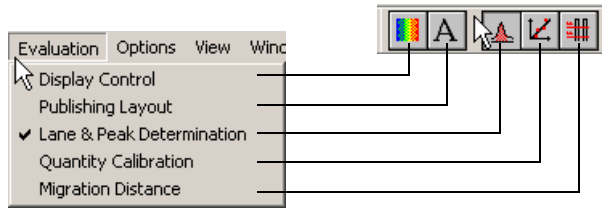


Figure 2-51 Mapping between Evaluation Menu Entries and Toolbar Buttons

Lane & Peak Determination Toolbox

The controls of the **Lane & Peak Determination** toolbox allow you to define lanes (profiles) and peaks on the image for signal intensity evaluation.

To display the Lane & Peak Determination toolbox:

• On the main menu, click **Evaluation** and choose **Lane & Peak Determination**.

Alternatively you can click the **Lane & Peak Determination** button on the toolbar.

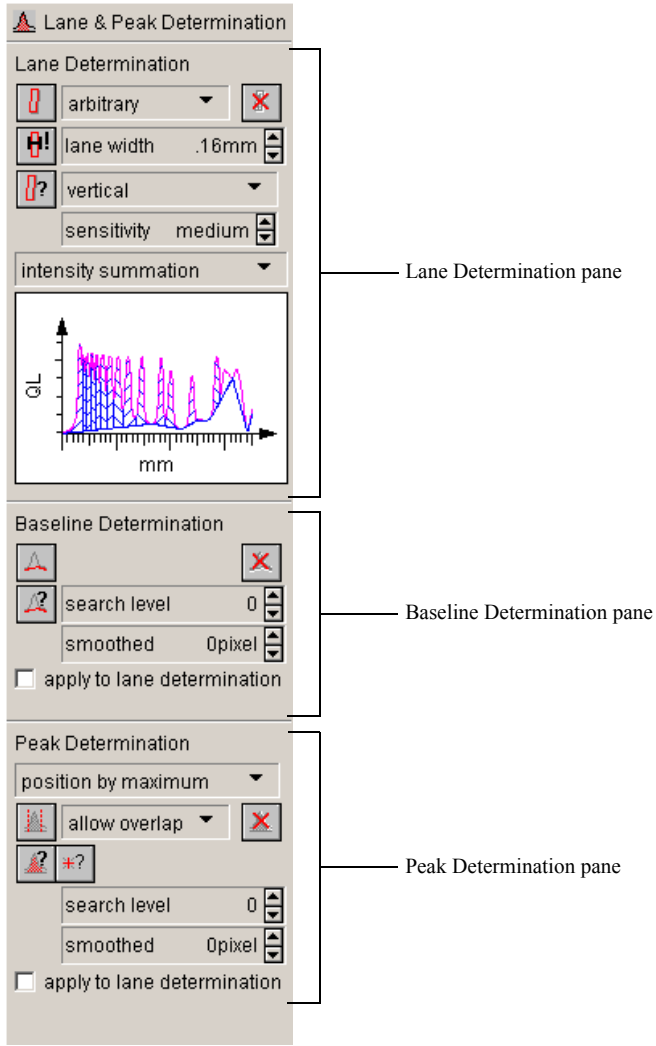


Figure 2-52 Lane & Peak Determination Toolbox (Overview)

Lane Determination Pane

The first step in the analysis of a 1D-application is the determination of the lanes.

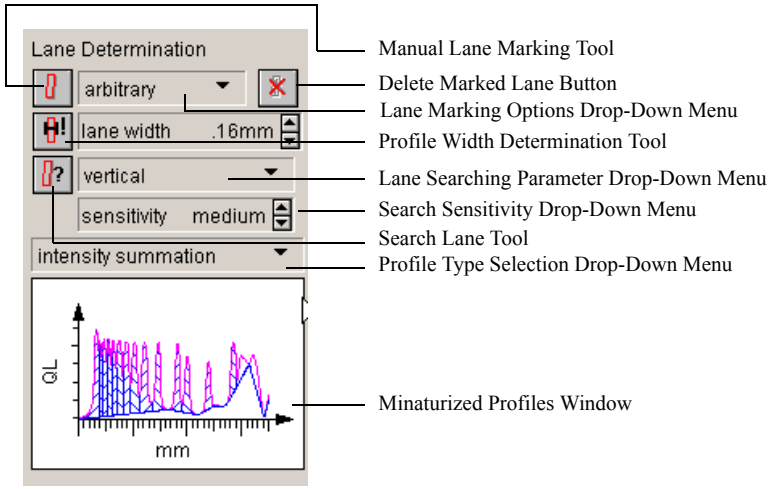


Figure 2-53 Lane Determination Pane (Overview)

Manual Lane Marking Tool: Select this tool to enter the manual lane marking mode. The **Lane Marking Options** drop-down menu next to the button offers the following lane marking options:

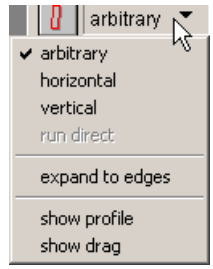


Figure 2-54 Lane Marking Options Drop-Down Menu

Densitometer windows can be drawn **Arbitrary** (in any angle), **Horizontal**, or **Vertical**. **Expand Lanes to Edges** allows the densitometer windows to go from top to bottom of the image. **Show Profile** opens the **Profiles** window directly after the densitometer window has been drawn.
If the **Show Drag** option is turned on, the profile of the currently dragged densitometer window is continuously updated in the miniaturized profiles window of the toolbox.

Delete Marked Lane Button: Use this button to delete a previously marked lane.

Profile Width Determination Tool: This tool is used to enter the width of the densitometer window in the currently selected unit of measurement (pixels, cm, or inch). The width should be selected to enclose the entire lane without too much extra space.

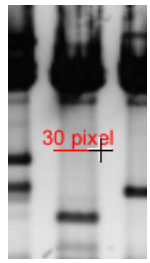


Figure 2-55 Determinating Profile Width

Search Lane Tool: Clicking this button activates the lane search mode. The above-mentioned options **Horizontal**, **Vertical** and **Expand to Edges** can be selected from the adjacent **Lane Searching Parameter** drop-down menu.

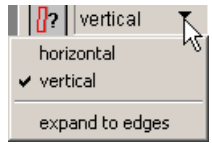


Figure 2-56 Lane Searching Parameter Drop-Down Menu

The miniaturized profiles window allows you to display the **Intensity Summation**, the **Averaged Intensity** and the **Optical Density** for a selected lane. To choose the desired graph, use the **Profile Type Selection** drop-down menu on top of the diagram.

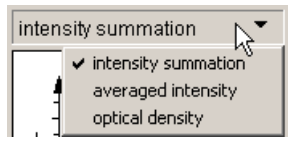


Figure 2-57 Profile Type Selection Drop-Down Menu

The diagram shows the selected parameter (y-axis) plotted against the distance unit selected in the **Image Attributes** dialog box of the **Option** menu.

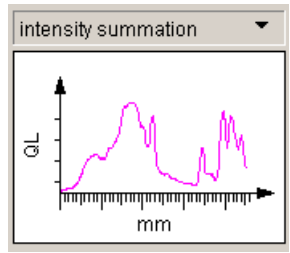


Figure 2-58 Miniaturized Profiles Window

Baseline Determination Pane

This pane will be needed only for the quantification of the bands after standardization.

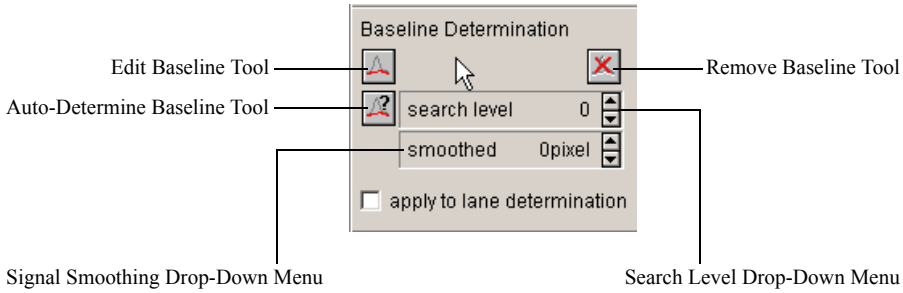


Figure 2-59 Baseline Determination Pane (Overview)

Edit Baseline Tool: Clicking this button activates the Edit Baseline function. If no baseline was determined yet, a straight line is drawn from the leftmost intensity to the rightmost intensity of the profile. This line can be edited in the **Profiles** window by moving the handles attached to the line. Double-clicking on any position of the baseline creates a new handle.

Remove Baseline Tool: This button removes the baseline(s) of the currently selected profile(s).

Auto-Determine Baseline Tool: This tool allows you to determined the baseline automatically. The **Smoothed** parameter should be set to a value of 2 or higher to omit single pixel noise and the **Search Level** can be set to various values reaching from (0 – insensitive, to 10 – extremely sensitive).

After optimizing all parameters for an example lane, you can select the **Apply To Lane Determination** checkbox to use the settings for any other lane to be selected.

Peak Determination Pane

This part of the toolbox is used for the determination of peaks.

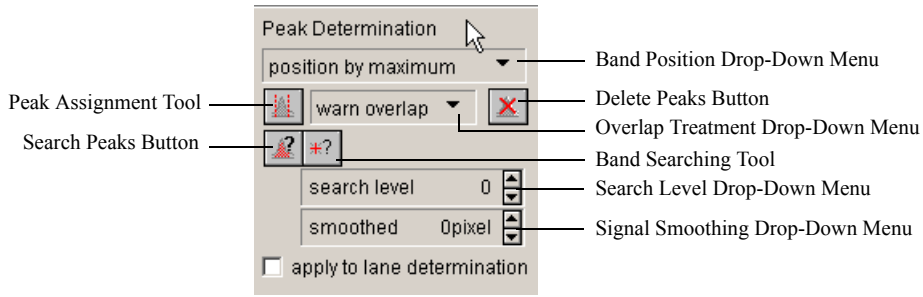


Figure 2-60 Peak Determination Pane (Overview)

Band Position drop-down menu: Use this drop-down menu to mark the position of the band by **Position by Maximum** or **Position by Center**.

Peak Assignment Tool: This button is used for manual peak assignment. Click on the start of the peak in the image or in the profile window, press the mouse button and drag the second mark to the end of the peak. You can change the peak region by dragging one of the marks. After the peak region is properly defined, right-click or press the ENTER key to confirm.

Overlap Treatment Drop-Down Menu: The drop-down menu next to the peak assignment tool is used to define AIDA's action if an overlap of peak regions is detected:

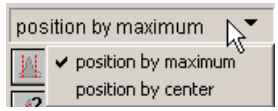


Figure 2-61 Overlap Treatment Drop-Down Menu

The options **Allow Overlap**, **Avoid Overlap** and **Warn Overlap** are available. Selecting **Avoid Overlap** will turn off the overlapping of regions by either keeping peaks separate or by merging two strongly overlapping peaks, after the region is confirmed.

Better results for quantification are obtained by using the manual peak assignment mode in the profile or image window.

Delete Peaks Button: Click this button to delete all currently selected peaks.

Search Peaks Button: Select this button after setting the **Search Level** and **Smoothed** parameters to search peaks automatically within the active profile(s).
Although these parameters have the same names as the previously described ones, setting the same values for the different functions will not give the same results. All these parameters should be tested for a particular image type before they are applied to an actual image of the tested type. The selected parameters can be saved under specific names using the **Preferences** command of the **Options** menu. Clicking the **Apply To Lane Determination** box enables the immediate use of the selected parameters for the evaluation of the following densitometer windows.

Band Searching Tool: Use this button to search for individual bands by clicking on them. Bands that are not found with any set of parameters can be double-clicked when this function is activated to obtain migration distance analysis for them.

Quantity Calibration Toolbox

The controls of the **Quantity Calibration** toolbox allow you to perform quantity calibrations of 1D densitometers by assigning amount standards to the bands.

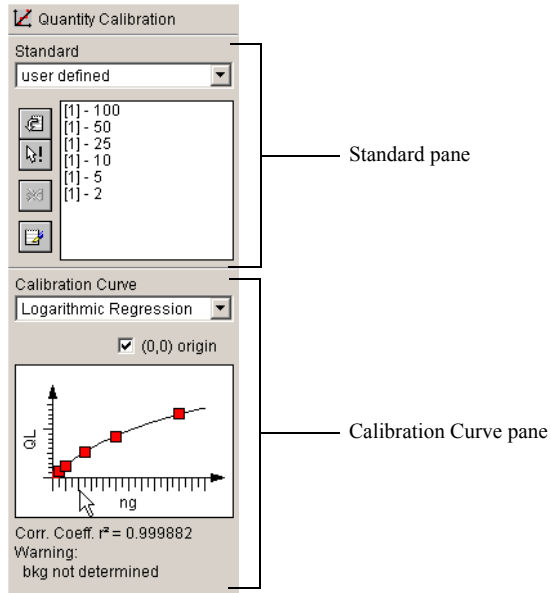


Figure 2-62 Quantity Calibration Toolbox (Overview)

To display the Quantity Calibration toolbox:

- On the main menu, click **Evaluation**, and then click **Quantity Calibration**.

Alternatively you can select the **Quantity Calibration** button on the toolbar.

Standard Pane

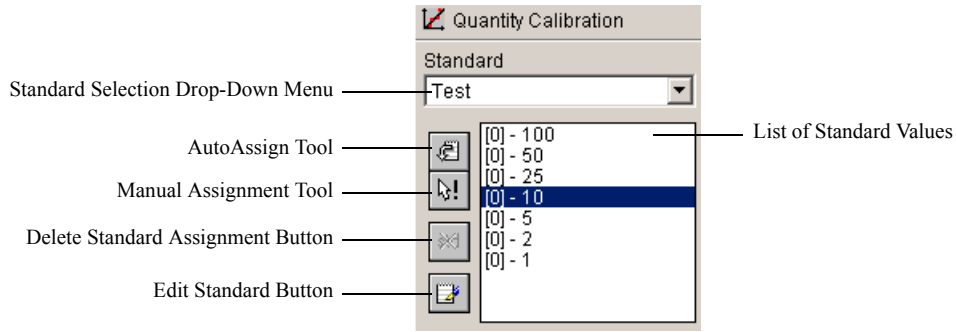


Figure 2-63 Standard Pane (Overview)

Manual Assignment Tool: This button allows you to assign amount standards manually to the bands by entering appropriate values via a dialog box.

AutoAssign Tool: Use this button to semi-automatically apply Standard values to bands one by one using a predefined list. The predefined list can be selected from the **Standard Selection** drop-down menu. The specified standard values are displayed in the **List of Standard Values** pane below the drop-down menu.

Delete Standard Assignment Button: Using this button you can delete selected standard assignment(s).

Edit Standard Button: Use this button to enter and edit predefined standards. Clicking this button opens the **Quantity Standards** dialog box.

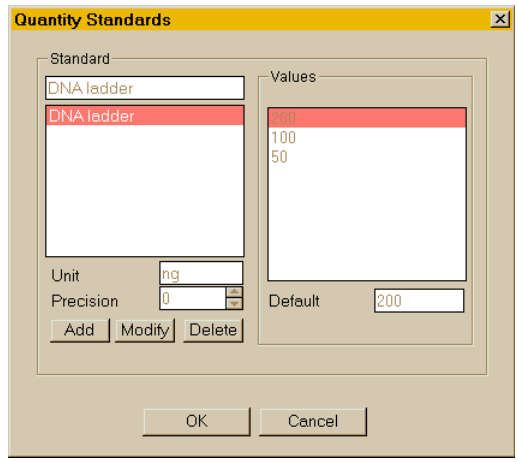


Figure 2-64 Quantity Standards Dialog Box

In the **Quantity Standards** dialog box the **Standard** name and **Values** are entered and the dimension of the amounts can be selected.

The **Add**, **Modify** and **Delete** buttons are used to alter the list of standards.

All standards defined should be saved for future use. This is also true for one-time standards because evaluation get frequently re-done, which means that the standard values are lost unless entered in the list.

Calibration Curve Pane

In the **Calibration Curve** pane of the **Quantity Calibration** toolbox the mathematical calculation of the standard curve can be selected and the result is displayed in the graph at the lower end of the toolbox.

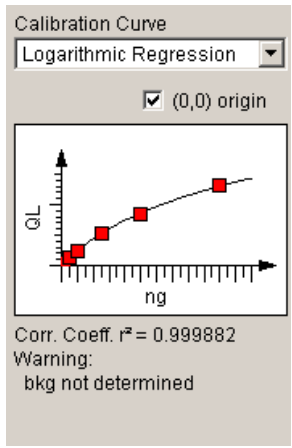


Figure 2-65 Calibration Curve Pane

The **Calibration Curve** drop-down menu provides the following functions:

- Linear Interpolation
- Linear Regression
- Logarithmic Regression
- Log. Weighted Regression

Linear Interpolation calculates lines to join each data point.

Linear Regression optimizes a linear graph so that the deviation of the actual points from the curve is minimized.

Logarithmic Regression is used to determine the standard curve with logarithmic characteristics, which is found in many saturating effects (e.g. absorption).

Log. Weighted Regression can be described as linear regression using a double logarithmic scaling. The result is a better optimization of the curve fit for the smaller values, instead of a better fit for the higher values (**Linear Regression**).

Click to select the **Origin** option to force the curve through the origin.

The correlation coefficient (**Corr. Coeff.**) of the estimation is shown below the curve. A value of 1 identifies a perfect correlation, a value of 0 indicates full independence of values. Good standard curves should have value very near to 1.

Double-clicking on the graphics pane opens the **Quantity Calibration** window (see the “Quantity Calibration Window” section of this manual for details).

Sometimes a warning will be displayed below the graphics pane, stating 'bkg not dertermined', to indicate that no background region has been assigned yet. It can also indicate the lack of a local background for some regions.

Migration Distance Toolbox

This toolbox is used to enter the values for the bands in the standard lanes. The resulting calibration curves are subsequently used for the determination of unknown bands.

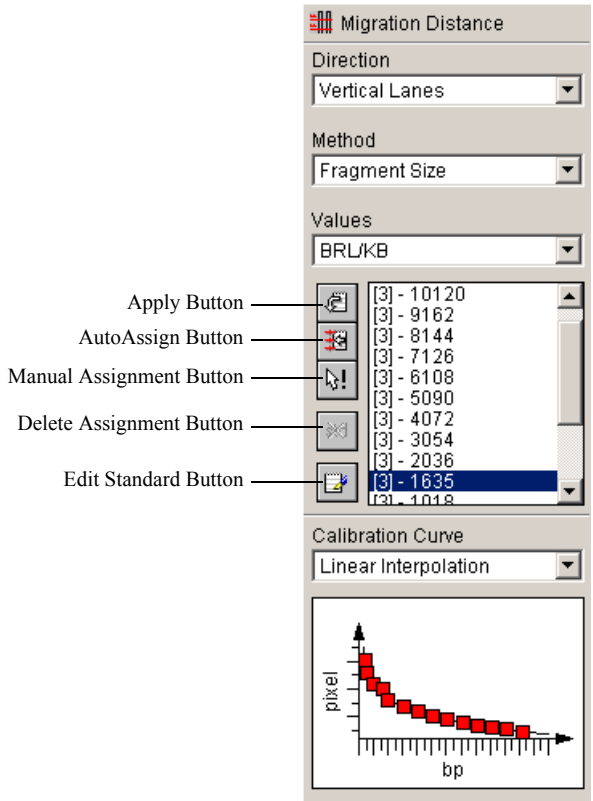


Figure 2-66 Migration Distance Toolbox (Overview)

The **Direction** drop-down menu is used to select the lane type used for the calibration (**Vertical Lanes** or **Horizontal Lanes**).

The **Method** drop-down menu is used to select the Migration Distance determination method (**RF-Value**, **Fragment Size**, **Molecular Weight**, or **PI-Value**). Choosing one of this methods will call predefined standard lists and calibration curve functions associated with the selected method.

The predefined standard is selected from the **Values** drop-down list. The buttons on the left side of the standard value window are used to assign the underlined value in the list to an assigned band in the image or in the profile by clicking on it.

Apply Button: This button applies the current value to a band.

AutoApply Button: This button assigns the currently underlined value to the clicked band and all values above and below to the bands that are marked above and below of this band, resulting in an automatic assignment of all standard bands at ones.

Manual Assignment Button: This button is used to manually assign a value that is not part of a predefined standard to a clicked band.

Delete Assignment Button: This button deletes all selected standard assignments.

Edit Standard Button: This button is used to edit predefined standards. After clicking this button a dialog box appears allowing you to add, modify or delete standards.

Only standards for the currently selected **Method** in the **Migration Distance** toolbox can be set in the **Standard** pane. Click the **Modify** button to get access to the currently selected scheme. Click **Delete** to erase the current standard value list or **Add** to enter a new list of standard values. The **Standard** name, **Values**, **Unit**, **Precision** (digits after the comma) and **Default Value** (the selected default value in the list) can be entered. The **Default Value** should be set to the value of the band that is easiest to spot in the standard lane.

In the lower part of the **Migration Distance** toolbox the **Calibration Curve** function is selected. The method of standard curve determination can be choosen from the predefined list. Usually the default function works best. The following functions are available depending on the selected **Method:**

- **None** – this option will shut off the background calibration e.g. if this function is too time consuming during editing of the standards
- **Linear Interpolation**
- **Linear Regression**
- **Logarithmic Interpolation**
- **Logarithmic Regression**
- **Hyperbolic Regression**
- **Hyperbolic Interpolation**

You can set your favorite calibration function as default by saving the current settings using the **Preferences** dialog box of the **Options** menu together with all other variables.

Publishing Layout Toolbox in 1D Evaluation

In AIDA 1D Evaluation the standard AIDA **Publishing Layout** toolbox is expanded by three additional buttons for specifying the print options for the **Profiles**, **Quantity Calibration** and **Runlength Calibratio**n:

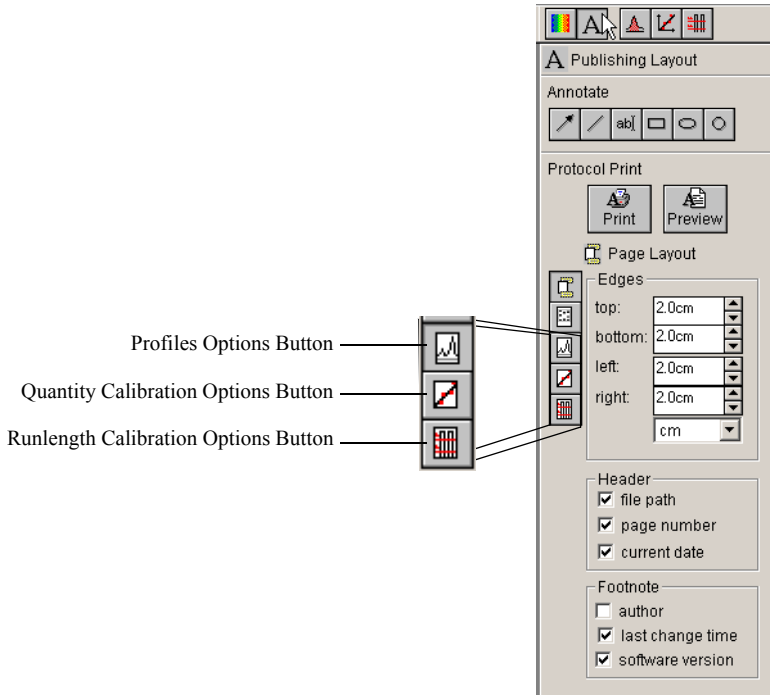


Figure 2-67 Publishing Layout Toolbox (Overview and Toolbar)

Print Options for Profiles

Using the **Profiles Options** button you can configure layout setting for the **Profiles** window.

1 Click the **Profiles Options** button.

The appearance of the **Protocol Print** pane changes.

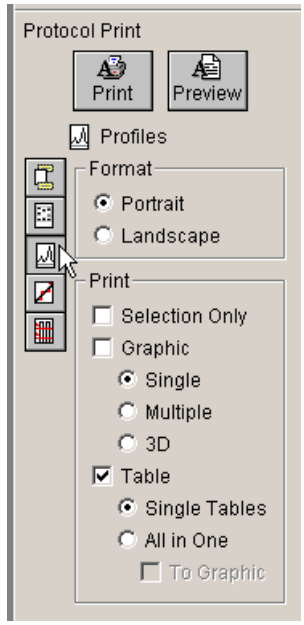


Figure 2-68 Print Options for the Profiles Window

2 Select the appropriate options:

In the **Format** pane select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button.

In the **Print** pane the scope for the print output can be specified:
Click **Selection Only** to print only the densitometers that are currently selected. **Graphic** selects the graphical representation of multiple selected profiles: **Single** shows each densitometer curve in a separate graphic, **Multiple** shows all (selected) curves on top of each other in a single graphic, and **3D** shows all (selected) curves in a 3-dimensional fashion within the same graphic.

To turn on printing of the result tables for the quantification and/or evaluation of the migration data, click to select the **Table** box. **Single Tables** prints a separate table for each 1D-densitometer, **All in one** prints all results combined in a single table. **To Graphic** prints the tables together with its associated densitometer graphic on a single page.

Print Options for Quantity Calibration

The second additional button of the **Publishing Layout** toolbox is used to configure the layout setting for the **Quantity Calibration** window.

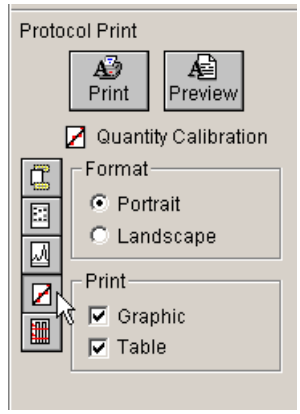


Figure 2-69 Print Option for the Quantity Calibration Window

As for the **Profiles** you can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** pane. The options in the **Print** pane enable or disable the printing of the calibration curve (**Graphic**) und/or the calibration table (**Table**).

Print Options for Runlength Calibration

The last button of the **Publishing Layout** toolbox is used to configure the layout setting for the **Runlength** window in the **Protocol Print** pane.

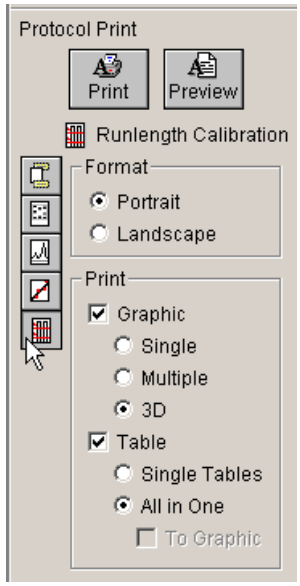


Figure 2-70 Print Options for the Runlength Calibration Window

As for the **Profiles** you can select the paper orientation (**Portrait** or **Landscape**) by clicking the appropriate radio button in the **Format** pane.The options in the **Print** pane enable or disable the printing of the runlength calibration curve(s) (**Graphic** checkbox). Clicking **Single** shows each runlength calibration curve in a separate graphic, **Multiple** shows all curves on top of each other in a single graphic, and **3D** shows all curves in a 3-dimensional fashion within the same graphic.

To turn on the printing of the runlength calibration data, click to select the **Table** box.

Single Tables prints a separate table for each calibration data set, **All in One** prints all results combined in a single table.

Print

In AIDA there are two ways for printing your image and evaluation data:

- Choose **Print** from the **File** menu,

  or

- Use the **Protocol Print** pane on the **Publishing Layout** toolbox.

The **Print** command of the **File** menu allows you to print your data 'on the fly' without permanently storing the layout/printing settings, whereas the **Protocol Print** pane provides you with a complete set of printing features which includes saving the setting with the data file for reasons of reproducibility.

Choosing the **Print** from the **File** menu opens the **Print** dialog box, allowing you to configure the printout to contain only the overlays, results and graphs that are currently needed. Click the checkboxes for the various items to turn them on or off. Select the number of copies, you want to print.

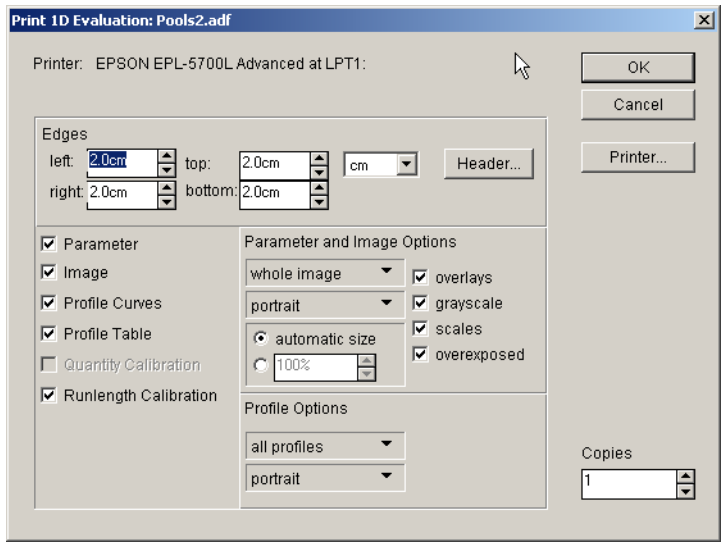


Figure 2-71 Print Dialog Box

The margins of the printout can be selected in the **Edges** part of the dialog box. The **Header** button displays the following dialog box:

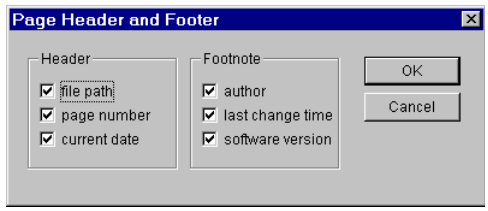


Figure 2-72 Page Header and Footer Dialog Box

Here the **Header** and **Footer** of the report can be configured to contain various image and evaluation specific data.

The **Parameter** (data overlays), the **Image**, **Profile Curves**, **Profile Table**, **Quantity Calibration** and **Runlength Calibration** information can be selected in the left part of the **Print** dialog box.

In the right part of the dialog box (**Parameter and Image Options**) you can select **Whole Image**, or **Window Content** from the first drop-down menu. **Landscape** or **Portrait** format can be selected from the second drop-down menu. The printing of **Overlays**, **Grayscales**, **Scales**, and **Overexposed** can be selected. The printout can be automatically fitted to the page, or the relative size of the image representation can be selected (**in %**).

In the **Profile Options** pane you can specify to print the **Current Selection** or **all profiles** as well as the paper orientation (**Landscape** or **Portrait**) for the printout.

Preview

As for printing, in AIDA there are two ways of previewing pages before they are actually printed:

- Using the **Print Preview** option of the **File** menu, or
- Using the **Preview** button on the **Publishing Layout** toolbox.

The **Print Preview** option of the **File** menu opens the **Preview** dialog box, which allows you to specify the layout settings for the preview. The options presented here are the same as in the **Print** dialog box and those selectable from the various panes of the **Publishing Layout** toolbox. However, they are not stored with the data file.

The **Preview** option of the **Publishing Layout** toolbox allows you to preview all pages as configured in the **Publishing Layout** toolbox before they are actually printed. Clicking the **Preview** button opens the **Preview** window directly, since all layout setting are already made through the **Print Protocol** pane.

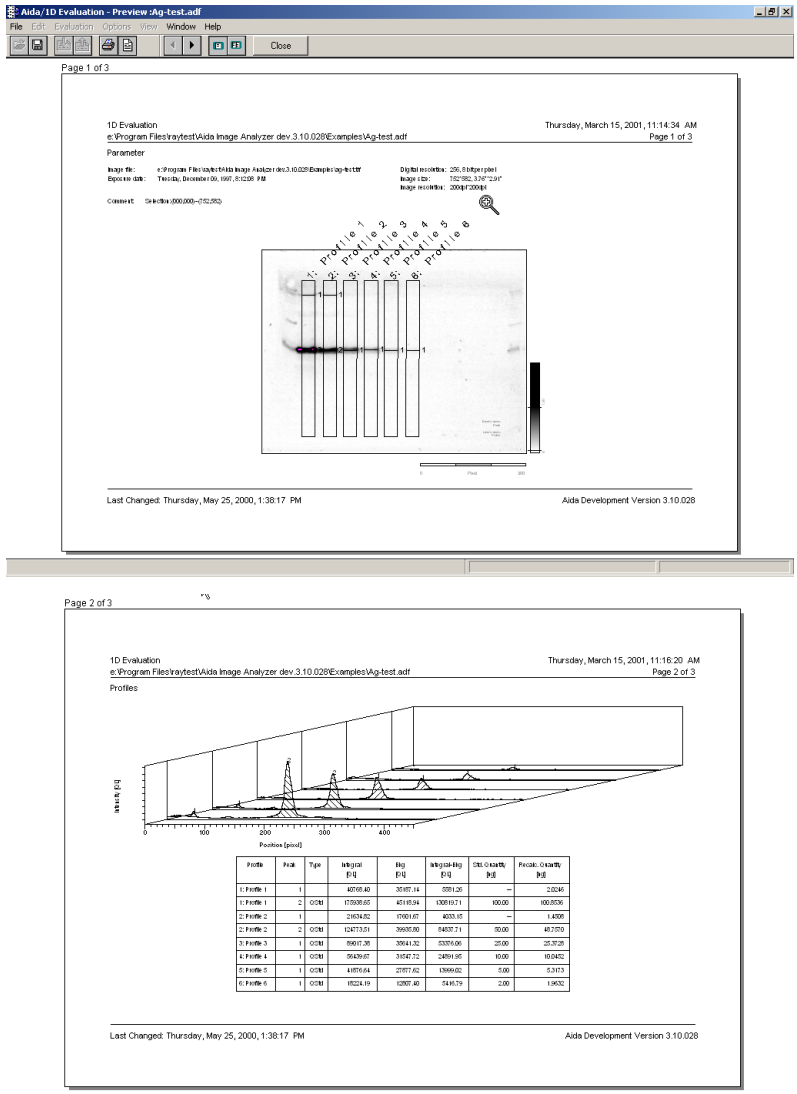


Figure 2-73 Preview Example Part 1

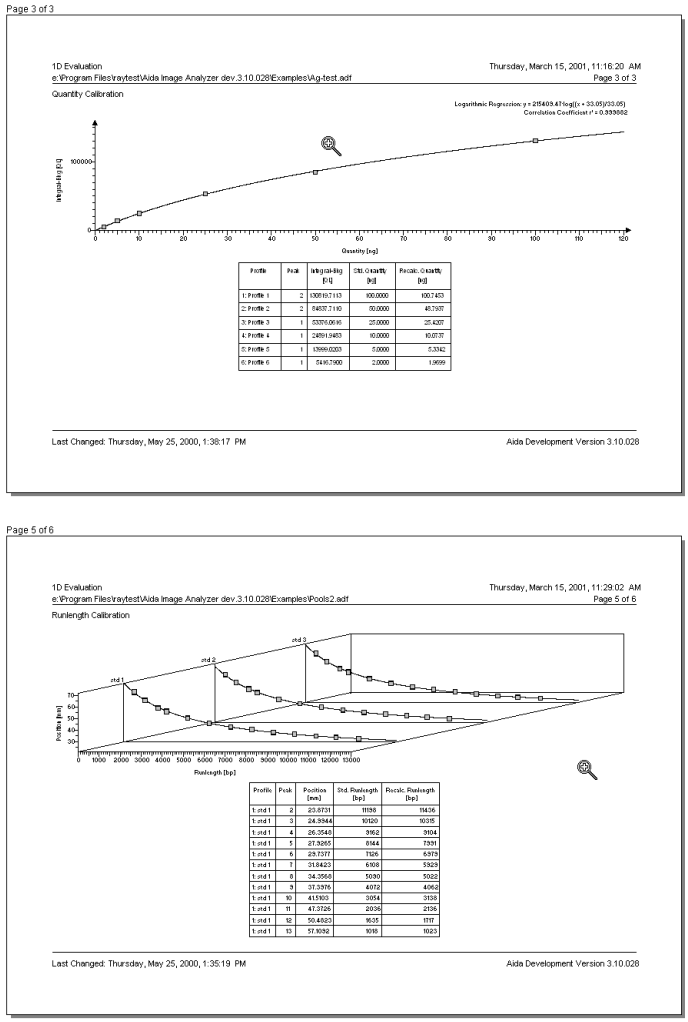

Page 3 of 3
1D Evaluation
Thursday, March 15, 2001, 11:16:20 AM
e:\Program Files\raytest\Aida Image Analyzer dev.3.10.028\Examples\Ag-test.adf
Page 3 of 3
Quantity Calibration
Logarithmic Regression: y = 215409.41*log((x + 33.05)/33.05)
Correlation Coefficient r² = 0.999992
Integral-Sig [IU]
Quantity [ug]
Profile | Peak | Integral-Sig [IU] | Std. Quantity [ug] | Recalc. Quantity [ug]
1: Profile 1 | 2 | 100119.7113 | 100.0000 | 100.7453
2: Profile 2 | 2 | 84637.7160 | 80.0000 | 85.7597
3: Profile 3 | 1 | 63259.0646 | 60.0000 | 65.4287
4: Profile 4 | 1 | 24591.9483 | 10.0000 | 10.0737
5: Profile 5 | 1 | 13988.0203 | 5.0000 | 5.3342
6: Profile 6 | 1 | 5416.2900 | 2.0000 | 1.9699
Last Changed: Thursday, May 25, 2000, 1:38:17 PM
Aida Development Version 3.10.028
Page 5 of 6
1D Evaluation
Thursday, March 15, 2001, 11:29:02 AM
e:\Program Files\raytest\Aida Image Analyzer dev.3.10.028\Examples\Pools2.adf
Page 5 of 6
Runlength Calibration
ctrl 1
ctrl 2
ctrl 3
Position [mm]
Runlength [bp]
Profile | Peak | Position [mm] | Std. Runlength [bp] | Recalc. Runlength [bp]
1: ctrl 1 | 2 | 23.8731 | 11196 | 11436
1: ctrl 1 | 3 | 24.3944 | 10120 | 10315
1: ctrl 1 | 4 | 26.3548 | 8162 | 8104
1: ctrl 1 | 5 | 27.9265 | 6144 | 7391
1: ctrl 1 | 6 | 29.7377 | 7126 | 6.975
1: ctrl 1 | 7 | 31.8423 | 5108 | 5929
1: ctrl 1 | 8 | 34.3566 | 5090 | 5028
1: ctrl 1 | 9 | 37.3976 | 4072 | 4062
1: ctrl 1 | 10 | 41.5103 | 3054 | 3138
1: ctrl 1 | 11 | 47.3126 | 2036 | 2136
1: ctrl 1 | 12 | 56.4823 | 1635 | 1717
1: ctrl 1 | 13 | 57.1092 | 1018 | 1023
Last Changed: Thursday, May 25, 2000, 1:35:19 PM
Aida Development Version 3.10.028

Figure 2-74 Preview Example Part 2

Export and Import

In AIDA 1D Evaluation various tables and graphs can be exported to be imported in other documents or programs. In addition the calibrations and templates can be exported to be applied to other images. The various functions can be found in the **Export** and **Import** submenus of the **File** menu.

Exporting Templates

To export your own evaluation templates:

1. On the **File** menu, click the **Export** menu item and then choose **Template** from the submenu.
2. In the **Export Template** dialog box that appears type a name for the export file and confirm the action by clicking **Save**,

or

1. Click the **Export Template** button on the toolbar to open the **Export Template** dialog box

Figure 2-75 Main Toolbar (Export Template Button)

2. Type a name for the export file in the **File Name** text box and complete the export action by clicking **Save**.

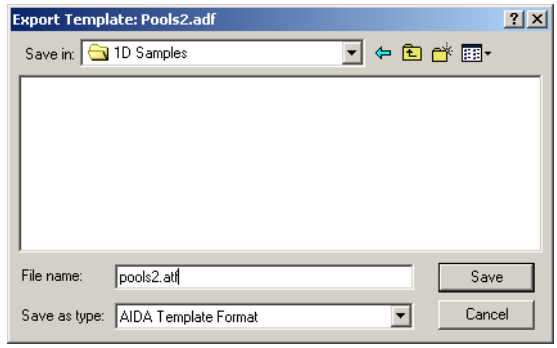


Figure 2-76 Export Template Dialog Box

Exporting Profiles

To export Profiles:

1 On the **File** menu, click the **Export** menu item and then choose **Profiles** from the submenu.

2 In the **Profiles Export** dialog box that appears type a name for the export file and specify the data format.

To export the graph in the Profiles window:

- Choose **Windows Metafile**.

  The graph is saved to a separate file in the Windows Metafile format.

To export the profile data of the Profiles window:

1 Select the **Profile Ascii File** data format.

2 Click **Save** to confirm the export.

  The **Profile Export** dialog box appears, allowing you to set further option for the export of the result table.

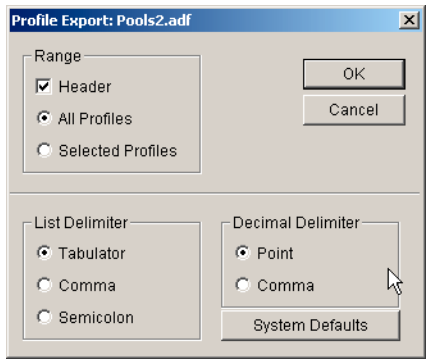


Figure 2-77 Profile Export Dialog Box

You can specify the **Range** of the exported rows (**Header** only, **All Profiles** or **Selected Profiles** only) as well as the **List Delimiter** and **Decimal Delimiter** in the respective panes of the dialog box. Selecting the **System Defaults** button applies the Windows default settings to **List Delimiter** and **Decimal Delimiter**.

3 Complete the action by clicking **Ok**.

  The data are saved to a simple ASCII (text) file.

Exporting 1D Report Table

To export the 1D Report Table:

1 On the **File** menu, click the **Export** menu item and then choose **1D Report Table** from the submenu.

2 In the **1D Report Table Export** dialog box that appears type a name for the export file.

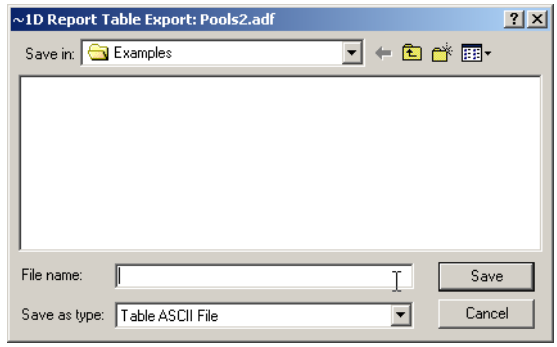


Figure 2-78 1D Report Table Export Dialog Box

3 Click **Save** to confirm the export.

The **Profiles Export Options** dialog box appears, allowing you to set further option for the export of the result table.

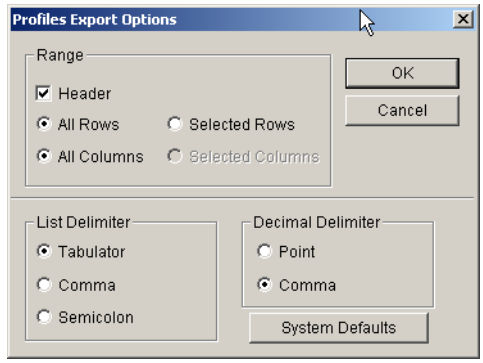


Figure 2-79 Profiles Export Options Dialog Box (1D Report Table Export)

You can specify the **Range** of the exported rows (**Header** only, **All Profiles** or **Selected Profiles** only) as well as the **List Delimiter** and **Decimal Delimiter** in the respective panes of the dialog box. Selecting the **System Defaults** button applies the Windows default settings to **List Delimiter** and **Decimal Delimiter**.

4 Complete the action by clicking **OK**.

The data are saved to a simple ASCII (text) file.

Exporting Quantity Calibration

To export Quantity Calibration Data:

1 On the **File** menu, click the **Export** menu item and then choose **Quantity Calibration** from the submenu.

2 In the **Quantity Calibration Export** dialog box that appears type a name for the export file and specify the data format.

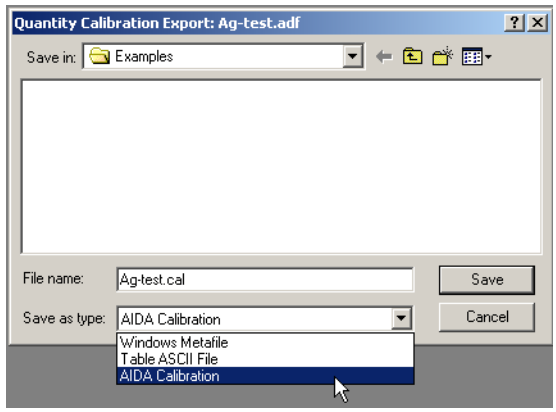


Figure 2-80 Quantity Calibration Export Dialog Box

To export the graph in the Quantity Calibration window:

- Choose **Windows Metafile**.

The graph is saved to a separate file in the Windows Metafile format.

To export the result table of the Quantity Calibration window:

1 Select the **Table Ascii File** data format.

2 Click **Save** to confirm the export.

The **Quantity Calibration Export Options** dialog box appears, allowing you to set further option for the export of the result table.

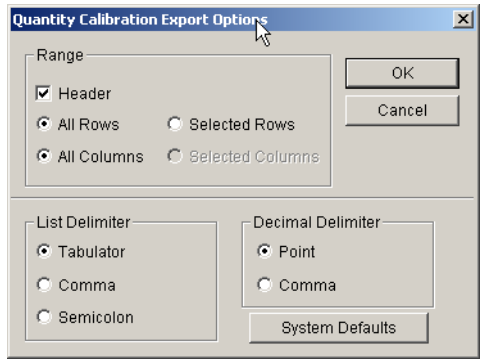


Figure 2-81 Quantity Calibration Export Options Dialog Box

You can specify the **Range** of the exported rows (**Header** only, **All Profiles** or **Selected Profiles** only) as well as the **List Delimiter** and **Decimal Delimiter** in the respective panes of the dialog box. Selecting the **System Defaults** button applies the Windows default settings to **List Delimiter** and **Decimal Delimiter**.

3 Complete the action by clicking the **OK** button.

The data are saved to a simple ASCII (text) file.

In addition the calibration data can be exported to an AIDA Calibration file that can be imported into another image.

Exporting Runlength Calibration

To export Runlength Calibration Data:

1 On the **File** menu, click the **Export** menu item and then choose **Runlength Calibration** from the submenu.

2 In the **Runlength Calibration Export** dialog box that appears type a name for the export file and specify the data format.

To export the graph of the Runlength Calibration window:

- Choose **Windows Metafile**.

The graph is saved to a separate file in the Windows Metafile format.

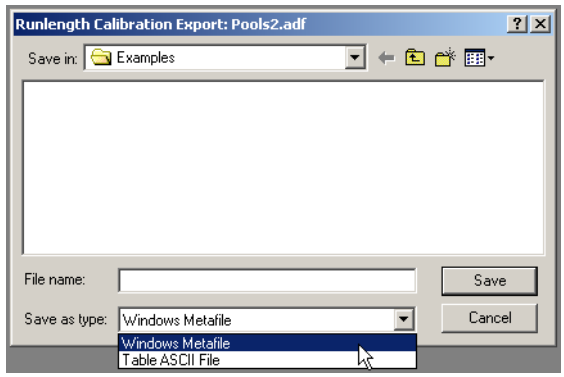


Figure 2-82 Runlength Calibration Dialog Box (Graph Export)

To export the result table of the Runlength Calibration window:

1. Select the **Table Ascii File** data format.
2. Click **Save** to confirm the export.

   The **Runlength Calibration Export Options** dialog box appears, allowing you to set further option for the export of the result table.

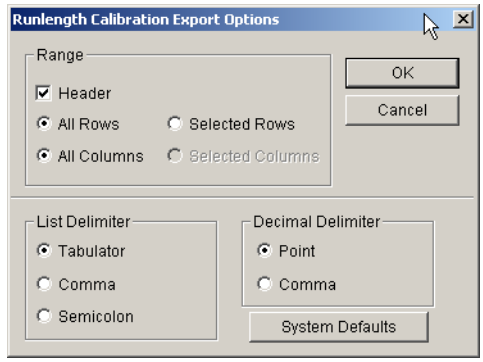


Figure 2-83 Runlength Calibration Export Option Dialog Box

You can specify the **Range** of the exported rows (**Header** only, **All Profiles** or **Selected Profiles** only) as well as the **List Delimiter** and **Decimal Delimiter** in the respective panes of the dialog box. Selecting the **System Defaults** button applies the Windows default settings to **List Delimiter** and **Decimal Delimiter**.

3 Complete the action by clicking **OK**.

The data are saved to a simple ASCII (text) file.

Importing Profile Templates

To import previously saved profile templates to another equivalent image:

1 On the **File** menu, click the **Import** menu item and then choose **Templates** from the submenu.

2 In the **Import** dialog box that appears select the template to be imported and click **Open**.

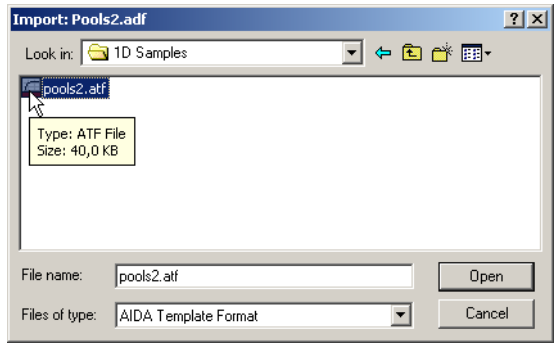


Figure 2-84 Import Template Dialog Box

The imported profiles can be arranged to fit the image by moving the whole array using the AIDA standard overlay editing functions.

Importing Quantity Calibration

1 To import the calibration curve from another image (e. g. taken at the same time with the same BAS screen, or from a second gel handled at the same time and captured under identical conditions), use the **Import** command of the **File** menu.

The **Quantity Calibration Import** dialog box appears allowing you to specify the calibration file (.cal).

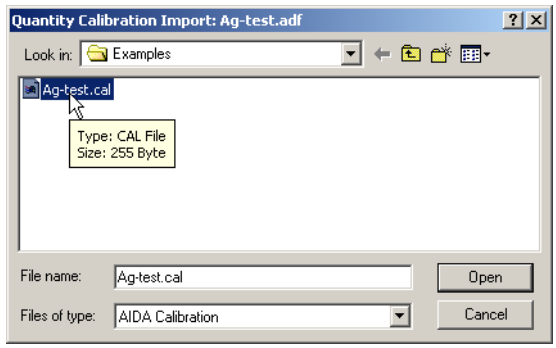


Figure 2-85 Quantity Calibration Import Dialog Box

Performing 1D Evaluations

3

Assigning Lanes and Bands

The first step in the 1D evaluation is the detection/marking of the lanes, which is followed by the detection/marking of the bands, normalized by the setting of the migration distance standards and the setting of the quantity standards, as applicable. The result is a fully evaluated image where all the information can be collected in a comprehensive printout.

With AIDA 1D Evaluation it is usually preferable to select all parameters that are necessary for an automatic detection of lanes, background and peaks first with one example lane. When all parameters are well chosen, further lanes can be automatically selected and assigned. The detected bands can than be normalized by entering the values for the standard lanes (quantification and migration distance standards).

To begin with the evaluation of lanes it is important to set the **Image Attributes** via the **Options** menu first (see “AIDA Image Analyzer Basic Concepts and Features User's Manual” for details). If these settings are not correct further image evaluation will not result in meaningful results.

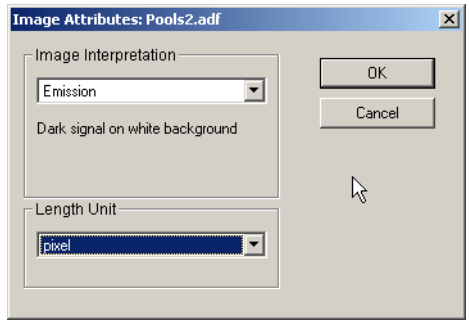


Figure 3-1 Image Attributes Dialog Box

When you use the **Lane & Peak Determination** toolbox for an application the first time, various parameters should be set:

1 Activate the **Lane Searching** function.

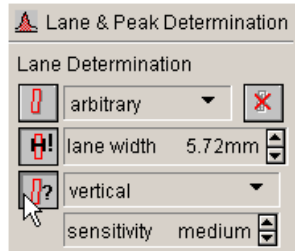


Figure 3-2 Activating Lane Searching

2 Click to the upper left corner of your lane, hold down the mouse button and drag a rectangle to the lower right of the lane.

3 Release the mouse button:

AIDA will search for the lane.

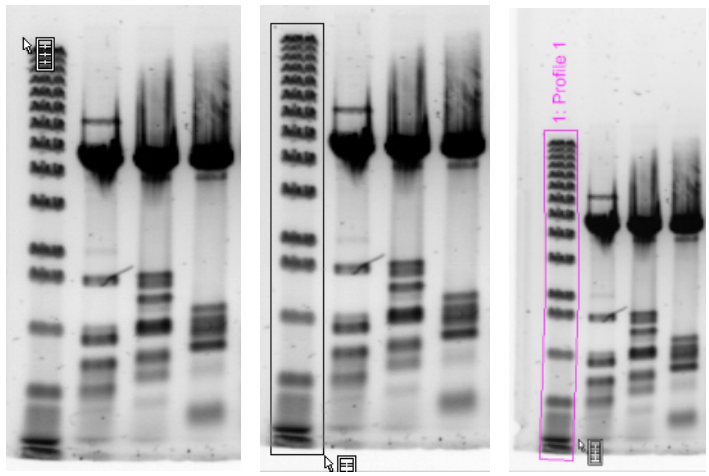


Figure 3-3 Semi-Automatic Lane Detection

4 Click the **Profile Width Determination Tool** and set the **Profile Width** to a value so that a little more than the band width is enclosed.

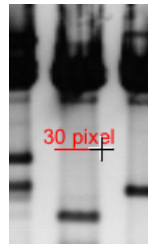


Figure 3-4 Setting Profile Width

5 Use the **Search Peaks button** to detect bands automatically.

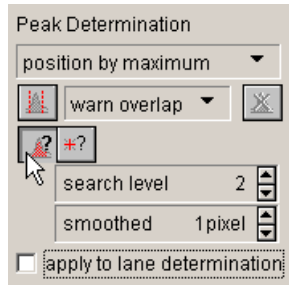


Figure 3-5 Activating Automatic Band Detection

If too many bands are found increase the **Search Level**. If too many artifacts are detected as bands, try to increase **Smoothed**. Do not forget to save your selected parameters under a **Preferences** scheme so that you can use them as a default for the next analysis. Once the parameters are selected appropriately the detection of lanes and bands should be fairly easy and straightforward.

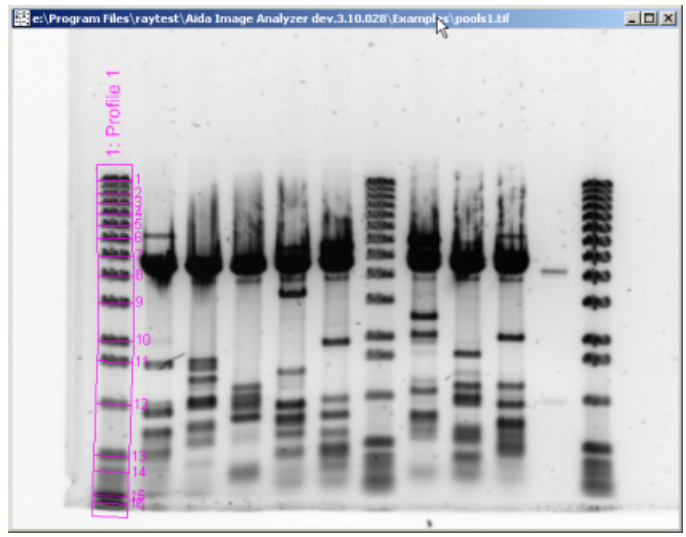


Figure 3-6 Automatic Band Detection Completed

6 Use the **Auto-Determine Baseline Tool** to draw a baseline.

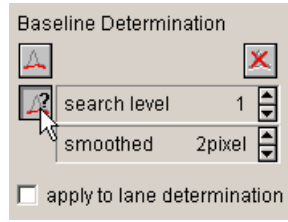


Figure 3-7 Activating Automatic Baseline Determination

If the baseline is too flexible and enters into the peaks you can increase the **Smoothened** value. If too much baseline is detected as peak you can increase the **Search Level**. If the baseline still does not fit your needs you can edit it manually by double clicking it in the **Profiles Window**.

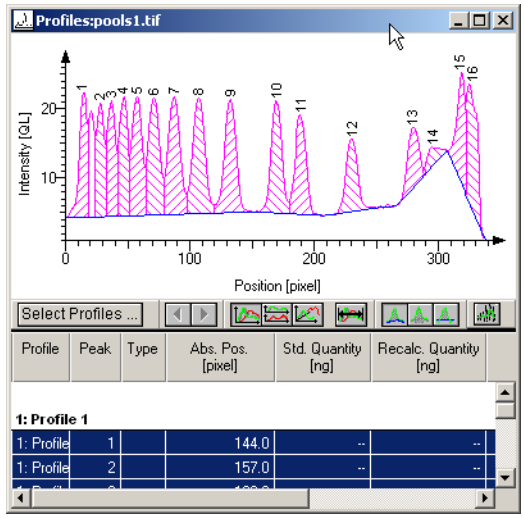


Figure 3-8 Automatic Baseline Determination Completed

All densitometer windows can be extended to the edges of the image (choose **Expand To Edges** from the contextual menu of the **Image** window) if necessary.

Multiple selections of profiles can be helpful for many manipulations and are achieved by opening a rectangle over all desired profiles or bands in the selection mode or clicking on each individual profile while holding down the CTRL key.

7 Finally assign bands within profiles that were not automatically detected by activating the **Band Searching Tool** and then clicking directly on the middle of the band.

8 Images where the automatic lane detection does not work can be assigned manually. Depending on the lane shape, the **Arbitrary**, **Vertical** or **Horizontal** densitometer function is activated.

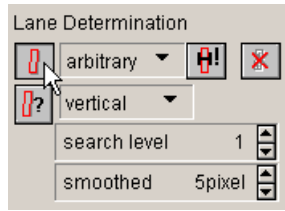


Figure 3-9 Activating Manual Lane Detection

a. Activate the **Manual Lane Marking Tool**.

b. Click in the middle of the lane to set the start point and drag the densitometer to the end of the lane.

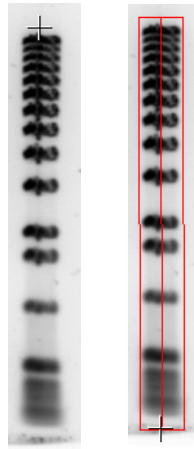


Figure 3-10 Manual Lane Marking 1

c. Double-click on a position in the frame to add a handle, which can be moved independently, enabling curved densitometer windows.

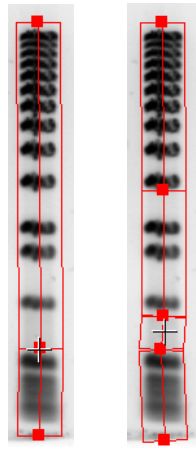


Figure 3-11 Adding Handles

9 Optimize search parameters for lanes, background and peaks with a representative lane.

10 Activate the **Apply To Lane Determination** function for all parameters to turn on automatic detection during the next lane assignment.

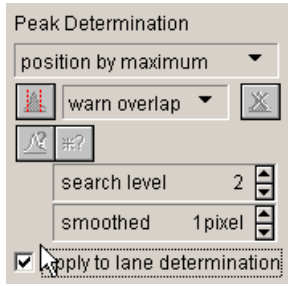


Figure 3-12 Enabling the Apply to Lane Determination Option

11 Detect all lanes by opening a rectangle over each lane (one by one) in the search mode .

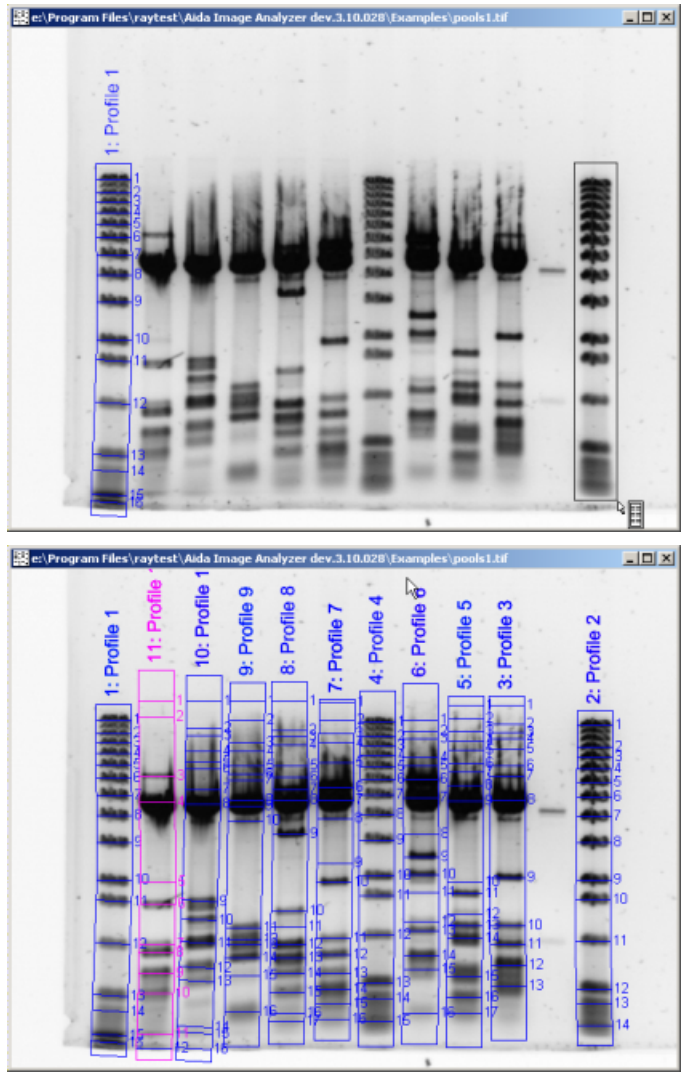


Figure 3-13 Automatic Detection of All Lanes

12 Delete peaks of no interest by opening a rectangle over them in the selection mode and pressing the DEL key.

Figure 3-14 Deleting Peaks

13 Check the **Profiles** window for peaks of interest.

14 Save the search parameters under a name specific for the source of the image (e.g. 'EtBr Gel') using the **Preferences** command of the **Options** menu.

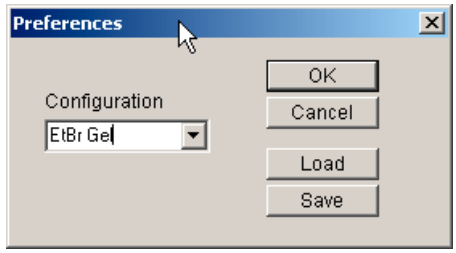


Figure 3-15 Saving Search Parameters

This allows you to simply reload them for the next image.

Assigning Migration Distance Standards

In all 1D applications the migration distance of a sample can be correlated with a physical constant of the sample. In the most widely used biochemical applications comparison is done according to size (molecular weight, fragment size), or charge (pI), or a combination thereof (RF). It should be noted that the computer and software are unable to detect the migration distance as such. They simply denote the absolute location of a band and compare it to the location of standard bands.

The evaluation is based on the image data in the lanes/densitometers and evaluated utilizing the **Runlength Calibration** data. Thus any tilt in the gel image and any smiling effect or distortion in the gel itself will hamper the evaluation. Since these conditions are frequently encountered in gels, blots or thin layer chromatography, a more elaborate standardization method should be employed. It is suggested to include at least two standard lanes (one on each side of the sample) and preferably a third one in the middle of the gel. This way the distortion of migration distance can be taken into account (see horizontal equimigration lines) and the most accurate results can be produced.

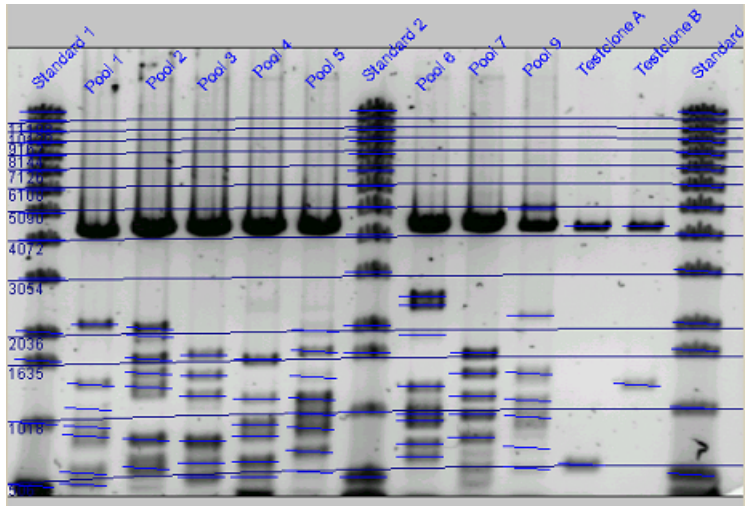


Figure 3-16 Migration Distance Example

When all bands have been assigned, the **Migration Distance** toolbox can be used to assign the standards.

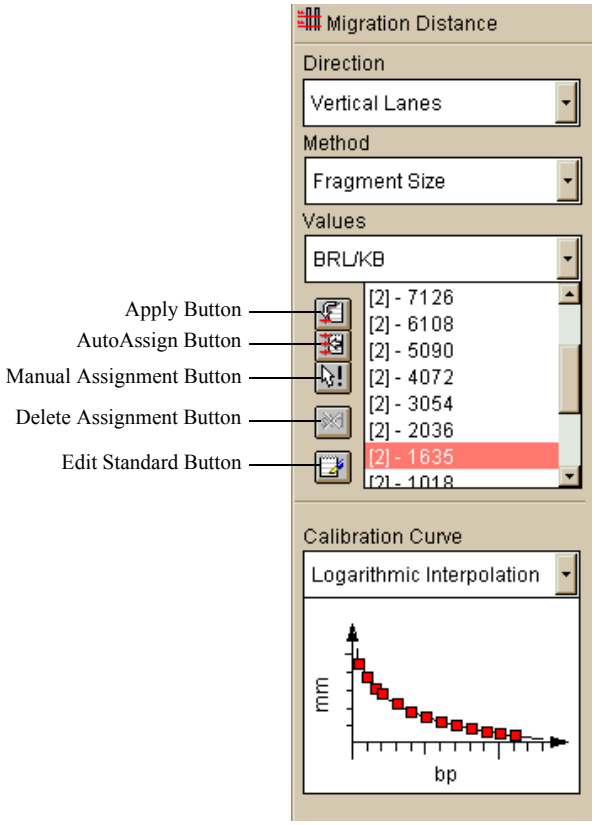


Figure 3-17 Migration Distance Toolbox

1 Select the **Method**, and **Values** of the standard.

All methods are automatically linked to the most suited evaluation function for the **Calibration Curve**. However, different functions can be selected to obtain a better fit of the calibration curve to the actual data.

You can automatically assign all values from the **List of Standard Values** (new standard lists can be easily edited) to the assigned bands:

2 Activate the **AutoAssign** tool and click on the band corresponding to the highlighted value on the list.

All other bands will be assigned accordingly. It should be checked that no band mentioned in the **Values** was unassigned in the image, so that the following band would get a wrong value.

3 The procedure should be repeated for all other standard lanes.

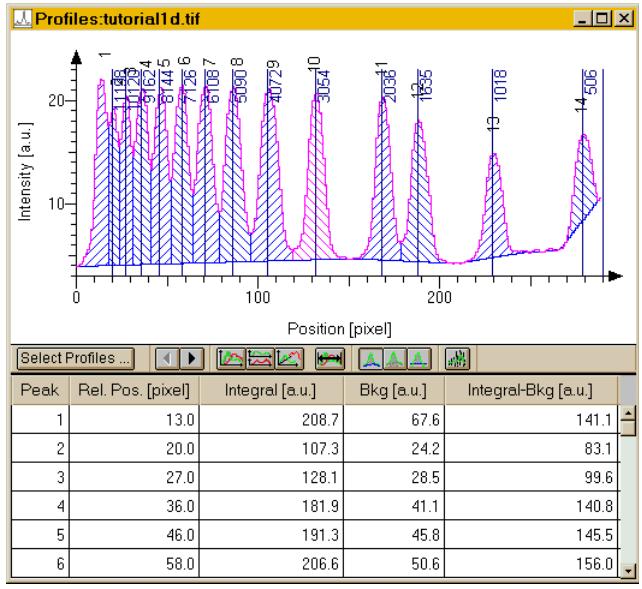
After all standard lanes are assigned, the normalization lines show the distortion effect in the current application. The fragment size of all assigned bands will be automatically deduced and receive a label indicating the result. The exact representation in the image depends on the **Image Overlays** settings (see the “Contextual Menu of the Image Window” section above).

You can print the image as well as the standard curve that was used to evaluate the run length data (a hyperbolic regression in this case) using the **Publishing Layout** toolbox controls as described in the respective section above. The evaluation of other physical parameters by the run length of samples in SDS polyacrylamide gels (kDa), isoelectric focusing gels (pI) and thin layer chromatography (RF values) can be performed analogously.

Assigning Quantitative Calibration Standards

As detailed in the introduction to this manual, the scientific relevance of the following quantification process has to be validated to obtain meaningful results. The example presented below is the fluorescence of ethidium bromide stained DNA. It has been established that the fluorescence resulting from ethidium bromide staining is proportional to the DNA amount (within certain ranges). The variation of fluorescence of different DNA fragments is not larger than the error of the whole procedure itself (less than 10 %).

The profiles determined by the **Lane & Band Evaluation** function can be visualized by activating the **Profiles** command of the **View** menu. Any profile can be selected using the **Select Profiles** pop-up menu, or using the arrows under the **Profiles** window.



Peak	Rel. Pos. [pixel]	Integral [a.u.]	Bkg [a.u.]	Integral-Bkg [a.u.]
1	13.0	208.7	67.6	141.1
2	20.0	107.3	24.2	83.1
3	27.0	128.1	28.5	99.6
4	36.0	181.9	41.1	140.8
5	46.0	191.3	45.8	145.5
6	58.0	206.6	50.6	156.0

Figure 3-18 Selecting Profiles

In the absence of a run length evaluation you will not see the deduced fragment length. If the bands can not be detected automatically, the profiles can be set manually.

After all peaks are properly determined, the quantity standards can be assigned:

1 Set the method for the intensity evaluation in the **Lane & Peak Determination** toolbox to **Intensity Summation**. **Optical Density** should be selected for color stained bands, **Intensity Summation** for radioactive, fluorescent stains and chemiluminescence applications, and **Averaged Intensity** can be used for fluorescent stains and chemiluminescence applications.

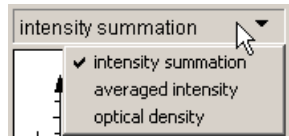


Figure 3-19 Setting the Intensity Evaluation Method

It should be noted that integration over the entire densitometer window should be done in sufficiently large densitometer windows to accommodate the entire band. Averaging can be performed best with densitometer windows that cover only the majority of the bands and omit empty space around them.

2 Assign bands representing a known quantity as Standards For Calibration By Selecting The **Manual Assigment Tool** from the **Standard** controls of the **Quantity Calibration** toolbox and click on the appropriate band in the image, or the peak in the profile.

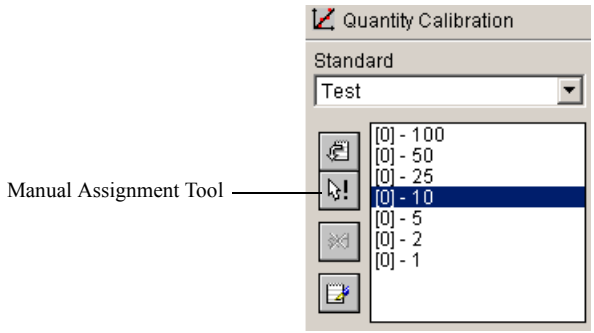


Figure 3-20 Manual Assigment Tool (Quantity Calibration Toolbox)

3 Enter the value and the dimension in the **Quantity Calibration** dialog box that appears.

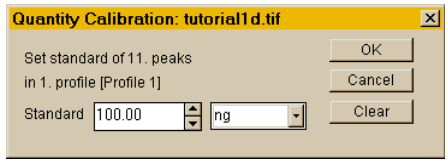


Figure 3-21 Quantity Calibration Dialog Box

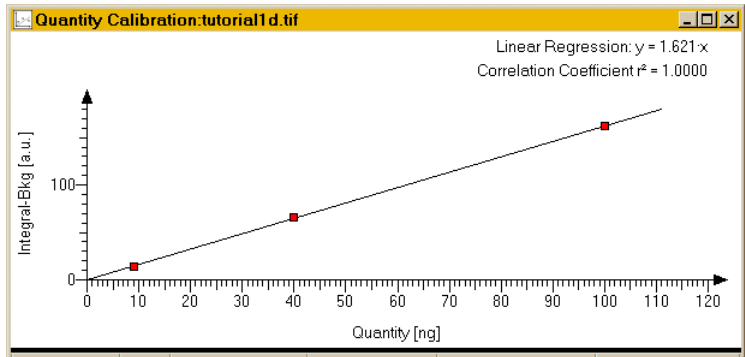
4 After all standards are entered, select the appropriate **Calibration Curve** type that fits the data (and application).

5 For most applications with properly assigned background, it might be advisable to select the **(0,0) Origin** option so that the standard curve goes through the origin.

6 Check the fit of the standard curve.

The r^2 value gives a clue about the fit; it should be very close to 1 for a good standard curve. The calibration curve can be selected on the basis of theoretical considerations or simply to fit the observed situation.

To evaluate the accuracy of the fit of the curve, you can check the difference between the quantity standard value and the recalculated standard value.



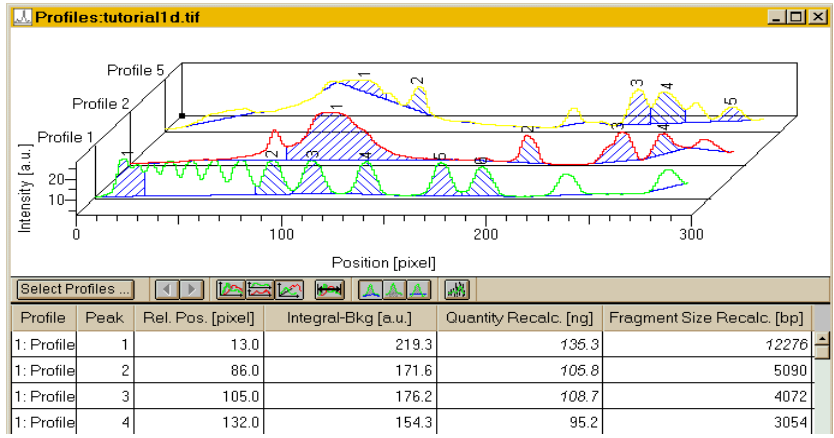
Profile	Peak	Integral-Bkg [a.u.]	Quantity Std. [ng]	Quantity Recalc. [ng]	Relative Error [%]
1: Profile 1	5	162.0	100.0	99.88	0.1158
11: Profile 11	1	65.4	40.0	40.34	0.8436
11: Profile 11	2	14.2	9.0	8.79	2.3641

Figure 3-22 Quantity Calibration Curve Example

Configuring the Evaluation Output

Following the calibration process outlined above, the entire image is calibrated and the results will be shown in the table pane of the **Profiles** window. One or more profiles can be displayed at the same time as outlined in the “Profiles Window” part of this manual.

It is important to use the **Migration Distance Normalization** function to get peaks of identical size at comparable positions.



Profile	Peak	Rel. Pos. [pixel]	Integral-Bkg [a.u.]	Quantity Recalc. [ng]	Fragment Size Recalc. [bp]
1: Profile	1	13.0	219.3	*135.3*	*12276*
1: Profile	2	86.0	171.6	*105.8*	5090
1: Profile	3	105.0	176.2	*108.7*	4072
1: Profile	4	132.0	154.3	95.2	3054

Figure 3-23 Migration Distance Normalization Example